SPAWAR
Systems Center
PACIFIC

Approaches for Evaluation of Episodic Discharges

A Review and Recommendations for Toxicity Testing Compliance Monitoring

Version 2

M. Colvin, G. Rosen, N. Hayman
SSC Pacific

B.C. Stransky
Amec Foster Wheeler

B. Phillips, B. Anderson
University of California, Davis

T. Hoang
Loyola University

H. Bailey, A. Cibor
Nautilus Environmental, LLC.

J. Diamond
Tetra Tech, LLC.

March 2018

EXECUTIVE SUMMARY

Industrial and municipal discharges are required to comply with increasingly stringent water quality requirements for stormwater runoff and other intermittent discharges. These requirements generally include end-of-pipe monitoring, enforced by National Pollutant Discharge Elimination System (NPDES) permits, prior to mixing in the receiving water. As a result, the existing EPA whole effluent toxicity (WET) test methods developed to assess continuous point source discharges (USEPA 1995, 2002 a, b, c) are now also being applied to episodic discharges, such as stormwater, dry dock discharges, and ballast water. These procedures have been criticized by the science community for failing to use exposures relevant to the intermittent, or episodic, nature of these events, instead using continuous static exposures (e.g., Burton et al. 2000, Diamond et al. 2006, Angel et al. 2010, Angel et al. 2015). Using WET testing procedures with continuous exposures for intermittent discharges likely overestimates potential toxicological effects in receiving systems. However, in some cases, short-term pulsed exposures to elevated chemical concentrations may also be more toxic than continuous exposure to an averaged exposure concentration (Reinert et al. 2002). Pulsed exposure toxicity regimes have been proposed as a method to make toxicity tests more representative of intermittent discharges (Brent and Herricks 1999, Diamond et al. 2006a, Gordon et al. 2012, Gosset et al. 2016). Several lab studies (primarily in freshwater systems and using agrochemicals) have explored the effects of several factors of pulsed exposures; these include pulse concentration, pulse duration, pulse frequency, latent effects, and age of exposed organisms. In addition, some frameworks have been suggested to utilize exposure regimes and methods in a regulatory setting.

Included herein is a discussion of the advantages of pulsed toxicity methodologies, and various considerations for designing pulsed exposure testing protocols. In addition, there is a brief discussion of potential options to incorporate pulsed exposure testing methods into regulatory frameworks. This review ends with implications for the current pulsed exposure work at SPAWAR Systems Center (SSC) Pacific and path moving forward.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS

ASTM	American Society for Testing and Materials
BMP	Best Management Practice
CORMIX	Cornell Mixing Zone Expert System
DGT	Diffusive gradient in thin film
DoD	Department of Defense
EC50	median-effect concentration
EPA	Environmental Protection Agency
ESTCP	Environmental Security Technology Certification Program
ETU	event toxicity unit
LC50	lethal-effect concentration
LET50	median lethal effect time
NBC	Naval Base Coronado
NBPL	Naval Base Point Loma
NBSD	Naval Base San Diego
NESDI	Navy Environmental Sustainability Development to Integration
NOEC	no observed effect concentration
NPDES	National Pollutant Discharge Elimination System
OF	Outfall
PAH	Polycyclic aromatic hydrocarbon
PE-LET50	post-exposure median lethal effect time
QSE	qualifying storm events
SERDP	Strategic Environmental Research and Development Program
SOP	Standard Operating Procedure
SPAWAR	Space and Naval Warfare Systems Center
TAC	Time-averaged concentration
TIE	Toxicity Identification Evaluation
TRE	Toxicity Reduction Evaluation
TST	Test of Significant Toxicity
USEPA	United States Environmental Protection Agency
WET	Whole effluent toxicity

1. INTRODUCTION

Industrial and municipal discharges are required to comply with increasingly stringent water quality requirements for stormwater runoff and other episodic (non-continuous) discharges. These requirements generally include end-of-pipe monitoring, enforced by National Pollutant Discharge Elimination System (NPDES) permits, prior to mixing in the receiving water. Regulatory concern with stormwater discharges is associated with the Clean Water Act's goal to prevent discharge of toxicants in toxic amounts (US EPA 1972). As a result, the existing EPA whole effluent toxicity (WET) test methods developed to assess continuous point source discharges (US EPA 1995, 2002a, 2002b, 2002c) are now also being applied to episodic discharges, such as stormwater. These industrial and municipal permittees, in fact, are frequently unable to comply with current stormwater NPDES requirements using current test methodologies, even with the implementation of rigorous Best Management Practices (BMPs; Katz et al. 2006). ~~30%~~Thirty percent of the samples tested from the study by Katz et al (2006) were acutely toxic at the end of pipe using a t-test. These samples were collected from multiple Naval Stations in San Diego during 11 storm events from 2002 to 2005. The current exceedance rate for acute toxicity at end of pipe Naval Base locations using the newer TST approach with allowance for a 50% effect in undiluted sample is approximately 20% depending on the specific location and year. Consistent acute toxicity exceedances at a few specific locations have recently resulted in the creation and implementation of Toxicity Reduction Evaluation Plans at Naval Base Coronado and Naval Base Point Loma in 2017. Every sample tested for chronic toxicity at the end of pipe during the past two years has resulted in a toxic response, while samples collected from adjacent receiving waters have been nontoxic.

Furthermore, passing or failing toxicity and chemical concentration limits in any given end-of-pipe stormwater discharge will vary depending on a multitude of factors such as the size and intensity of the storm, antecedent dry period, timing when the sample was taken, and type of sample (grab or composite) as shown effectively in pollutograph studies (e.g. Kayhanian et al. 2008). Timing and flows can also impact toxicity in typical wastewater discharges as well, but generally the chemical and physical properties of these flows will be much more uniform over time. These variables increase the complexity with regard to interpreting the results for a given end-of-pipe stormwater sample, necessitating a more realistic and standardized sample collection and toxicity exposure regime. The key element of exposure duration and integration with receiving waters is not adequately addressed using current methodologies to assess episodic discharges.

Toxicity tests are desirable because: 1) they take into account contaminant bioavailability in a sample, which can vary from the bioavailability in uncontaminated filtered water used to develop water quality criteria (Stephan et al. 1985); and 2) they incorporate the potential for adverse effects associated with exposure to complex mixtures (i.e. multiple contaminants), many of which may not be monitored. However, regulators require frequent monitoring of stormwater from end-of-pipe samples during the first flush (first few hours of rainfall) and evaluation of these samples with

laboratory toxicity tests using a continuous (i.e. static or static-renewal) exposure of up to 7 days, depending on the species and test endpoint. This methodology does not adequately replicate the dynamic nature of the stormwater exposure at either the point of compliance or as it mixes with the receiving environment (Katz et al. 2006; Stransky et al., 2015, Rosen et al. in press), which may have substantially different physical and chemical properties than the runoff itself. The limitations of using current WET methods for stormwater testing at end-of-pipe for compliance has been recognized by regulators, as reflected in a new NPDES Permit for Naval Base San Diego (NBSD; Permit R9-2013-0064). This permit includes a provision where a special chronic toxicity study may be conducted to propose modifications to the Water Quality Control Plan for the San Diego Region (Basin Plan) to incorporate mixing zone dilution credits, recognizing that end-of-pipe monitoring is not representative of actual conditions in the bay. Use of a more applicable and representative toxicity test method would provide a sound basis for supporting such a change.

A more realistic assessment of the toxicological impacts of stormwater runoff, or other episodic discharges (e.g. intermittent discharges of stormwater and relief water from dry dock outfalls at Naval shipyards, chlorinator/dechlorinator cooling water from pier side ship, etc.) on beneficial uses in the receiving waters is critical. This would support decisions related to the need and prioritization of appropriate Best Management Practices (BMPs), and meaningful compliance with Clean Water Act goals. Results from pulsed toxicity exposures have been well documented in the peer-reviewed literature as a means of improving the characterization of exposure and potential for toxicity associated with episodic contaminant exposures (Dupuis and Kreutzberger 2003, Butcher et al. 2006, Diamond et al. 2006a, Hoang et al. 2007a, 2007b, 2007c, Angel et al. 2010, Stransky et al., 2015) however, the development and application of standardized protocols that are accepted by the regulatory community are currently lacking.

It has been difficult to implement pulsed exposure methods in water quality management for several reasons (Gordon et al. 2012). Pulsed discharges result in varying exposure profiles to contaminants, thus more complex toxicological responses than might be expected with continuous exposures. In addition, there is currently no standardized method to define what a pulsed exposure might consist of. A few published methods proposed for pulses of specific chemicals or classes of chemicals include assessment metrics and subsequent management decisions based on mean exposure concentration, peak chemical concentration, mean test concentration, and median lethal time (Handy 1994, Brent and Herricks 1999, Gordon et al. 2012). In addition, depending on mode of action of the contaminant and/or the physiological response to toxicant stress in the target organism, the responses to pulsed exposures can vary dramatically. We discuss these topics in more depth in Section 33.

The intent of this review is to provide the reader with a background and quick reference to various consideration related to the design of pulsed exposure studies. The review first presents the generic benefits of pulsed exposure designs over traditional WET static exposure bioassays for intermittent discharges, focusing on stormwater. In addition, it explores several considerations of pulsed

exposure testing, using previous studies to determine how best to address each consideration, as well as options to implement pulsed testing are provided. Finally, special considerations for current projects, including those being conducted by the project team, are proposed.

Sources of literature for this review were obtained through key-word searches on publication databases for relevant experimental design and endpoints. Considerations of selected literature were made to include dose response data for the species evaluated and contaminants of concern that are relevant to DoD interests (e.g., trace metals and organics). Tables in section 3 were populated with studies that address the focus of each relevant sub-section.

1.1. Pulsed Exposure Testing Scenario and Current Limitations and Challenges

Following is a clear example of how WET testing is currently applied to episodic stormwater discharges in San Diego, CA and the limitations and concerns of current methods. The first NPDES Permit with a stormwater toxicity testing requirement was finalized in 2002 as Order No. R9-2002-0002, NPDES Permit No. CA0109363, for the Naval Base Point Loma (NBPL). Additional separate and similar NPDES Permits quickly followed and are in place for Naval Base San Diego (NBSD) and Naval Base Coronado (NBC). Each of these permits requires the collection and testing of stormwater from a number of locations for analysis of a suite of chemical constituents and toxicity.

Beginning with NBSD is November 2013, followed by NBPL in January 2015, and NBC in June 2016, new toxicity testing monitoring and data evaluation requirements have come into effect. In addition to acute toxicity testing at storm-water monitoring locations, a monitoring requirement to collect a paired sample for chronic toxicity at one storm-water monitoring location and associated receiving water location was added to each permit. For NBSD, stormwater monitoring locations and the receiving water are sampled once per semiannual period. For NBC and NBPL, stormwater monitoring locations and the receiving water are sampled twice per semiannual period. The new data evaluation process for compliance determination is the test of significant toxicity (TST). TST is a statistical comparison that reflects the survival in the stormwater sample vs a lab control. For routine acute toxicity tests, a TST result of "Fail" with an effect of 40% ~~percent~~ or greater requires follow up sample collection and testing during the next qualifying storm event. If the follow up sample results in a "Fail" with an effect of 20% ~~percent~~ or greater, a Toxicity Reduction Evaluation (TRE) Work Plan is required. For chronic toxicity, a TST result of "Fail" with an effect of 25% ~~percent~~ or greater in both the receiving water and storm water monitoring location samples requires a TRE Work Plan.

Currently 26 storm-water monitoring locations are sampled and tested for toxicity among the three Navy Base monitoring programs. Single grab samples are collected during qualifying storm events (QSEs). QSEs require an antecedent dry period of two to three days, and for samples to be within a 4-hour period of the start of discharge, or as soon as possible in the morning if discharge occurs at night outside of scheduled facility operating hours. Inevitably a wide range of conditions may

be captured in a particular grab sample depending on the storm characteristics, sampling location, sample timing, antecedent dry period, etc. These freshwater runoff samples are brought to the lab, salted up with artificial salts to marine conditions, and tested undiluted for a 96-hour period using mysid shrimp. A thorough review of the historical data has found few if any consistent patterns for both chemistry and toxicity. Between November 2013 and June 2017, 396 acute toxicity tests were performed where a TST results whereas reported by the toxicity laboratory. Of the 396 tests, 72 or 18% percent, have resulted in a compliance "Fail" in terms of being statistically different from the control. Thirty of the seventy-two "Fail" results have shown effects of 40% percent or greater.

At each of the three Bases, chronic toxicity testing was is performed at select representative outfall and receiving water locations using the sea urchin embryo development test based on preliminary 3-species sensitivity screening tests. Since chronic toxicity testing has been a requirement at each base, end-of-pipe storm-water samples have resulted in failures with greater than a 25% percent effect in 57% percent (4 of 7) samples at NBSD, 100% percent (9 of 9) samples at NBPL, and 100 % percent (4 of 4) samples at NBC. Conversely, during the same period, associated receiving water samples have exhibited toxicity in 0% percent (0 of 7) samples at NBSD, 0% percent (0 of 9) samples at NBPL, and 25% percent (1 of 4) samples at NBC.

Between 2002 and 2005 the Navy (SPAWAR SSC Pacific) conducted a thorough evaluation of toxicity of industrial storm-water discharges from U.S. Navy facilities bordering San Diego Bay (Katz et al., 2006). Similarly, this study found that end-of-pipe stormwater samples were acutely toxic to mysid shrimp and/or topsmelt minnows 30% of the time (15 of 51 first-flush samples tested). However, less than 1% of 202 receiving water toxicity tests exhibited toxicity to either of these two species.

Commented [HT1]: Should it be "silverside" instead on "minnows"

An additional special study conducted by SPAWAR in 2004 furthermore found both acute and chronic toxicity to multiple species in an end-of-pipe outfall, but no toxicity in tests conducted *in situ* in the immediate receiving water during a large storm event (Katz and Rosen 2004). The discrepancy between end-of-pipe test results and results in simultaneously collected samples in the immediate mixing zone is stark and suggests that current methods used for end-of-pipe testing are not representative of what is occurring in the adjacent receiving water.

Tests that fail toxicity triggers can lead to accelerated monitoring and potentially a Toxicity Reduction Evaluation (TRE) including Toxicity Identification Evaluations (TIEs). Subsequent steps may lead towards extremely costly capture and treatment solutions and/or lead to vulnerability of lawsuits. Efforts to reduce or eliminate toxicity are clearly important for areas with issues of real concern, but such triggers can also lead to misguided and costly approaches that fail to address meaningful impacts based on transient end-of-pipe results alone.

Stormwater monitoring for other locations in California have encountered similar challenges meeting Permit requirements at the end-of-pipe using existing methods. The State of California has been a leader with regard to Permits that require toxicity testing, but other States are also now

starting to incorporate similar requirements in select NPDES Permits (e.g. Washington, Virginia, Hawaii, and Florida). Many states without current stormwater toxicity testing programs have reached out to USEPA headquarters for guidance and are currently often referred to EPA Region 9 and the State of California given the progress in this region and lack of any unified guidance or framework at the national or state level (pers. comm., Laura Phillips, USEPA headquarters, State and Regional Branch ‖ Water Permits Division, Washington DC).

Commented [HT2]: ???

2. NEED AND ADVANTAGES OF PULSED EXPOSURE TESTING

Currently, whole effluent toxicity (WET) testing of end-of-pipe samples is the primary method used to determine discharge compliance with NPDES permits for industrial and municipal permittees. Despite their ubiquity, several researchers have noted a number of weaknesses associated with the use of current WET methods, and utility in determining if permittees are discharging toxic levels of toxicants to the receiving environment (Diamond and Daley 2000, Burton et al. 2000, Diamond et al. 2008, and Gosset et al. 2016). A primary weakness of current methods is that they do not use environmentally realistic exposure periods to discharge samples; instead determining exposure duration based on established test protocols, which focus on specific portions of a test species early life history.

2.

2.1. Increased Environmental Realism

A variety of NDPES permits require EPA WET methods to test for compliance with toxicity guidelines for episodic discharges, such as stormwater, intermittent dry dock discharges and discharges from submarine mounted dechlorination units. Standard methods do not account for discharge duration or dilution as these intermittent discharges enter the receiving environment, and the chemical and physical properties of the receiving environment (i.e. DO, hardness, salinity, pH) that may alter the susceptibility of organisms to toxicants present in the stormwater (Burton et al. 2000, Schiff et al. 2003, Diamond et al. 2006b, Gordon et al. 2012).

Existing WET test methods may ~~are~~ not appropriate for episodic discharges as the methods were originally designed to assess continuous point-source discharges (US EPA 1995, 2002a, 2002b, 2002c). For example, samples collected for the assessment of stormwater toxicity from industrial areas are frequently collected within the first few hours of an event (i.e. the "first-flush") and storm duration or 24-hour composite samples are often collected for watershed locations. Test organisms are then exposed to these samples for the entire duration of the toxicity test, which can range from 48 hours (acute ~~toxicity and short-term chronic~~ tests) to 7 days (short term chronic toxicity tests). This type of exposure might not be a realistic or representative of what is occurring at the point of compliance at the end-of-pipe nor in the receiving environment.

An evaluation of relationships between rainfall and runoff in highly urbanized environments (characterized by impervious surfaces) has found that runoff and discharge durations of stormwater can be predicted using rainfall data, which is more widely available (Katz et al. 2006, Colvin et al. in prep; Figure 2-1~~Figure 2-1~~). A recent analysis using rainfall data from 21 locations across the U.S. over the last 20 years found that, on average, 95% of storms resulted in 28 or fewer hours of discharge over a 96-hr period directly after the onset of the storm (Colvin et al. in prep). Exposing organisms to end-of-pipe samples for the entire 96-hr testing period is not appropriate as it does not characterize a relevant contaminant exposure regime. Furthermore, it fails to account

for changes in the sample properties as it mixes with receiving water environments which may have very different physical and chemical properties. Finally, for pulsed discharges a single sample will typically be collected, stored, and used for the duration of any tests, some which may require water renewals, as opposed to going out to obtain a fresh sample. Samples held in a refrigerator, or in a static test chamber, have the potential to degrade over time which also fails to replicate natural conditions.

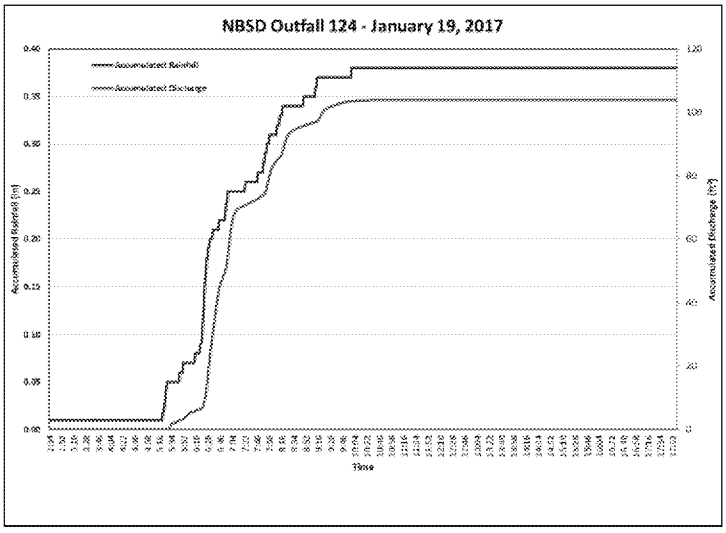

Figure 2-1. Accumulated rainfall and stormwater discharge from Outfall 124 at NBSDs on 19 Jan 2017. (From Colvin et al. in prep). Relationship between rainfall and discharge volume during one storm and resulting good correlation (R^2 = 0.9939) shows the appropriateness of using rainfall duration as a proxy for discharge duration.

3. Considerations of Pulsed Exposure Methods

A key proposed approach to address effects related to pulsed exposures will be to simply alter the exposure to a relevant time-period based on site-specific considerations (e.g. 95% runoff duration or the time to empty a dry dock). Following a pulsed exposure, the test organisms are then transferred to receiving water from the monitored location, or clean lab water if an adjacent receiving water is not available. Dilutions may be incorporated to add a level of realism that represents the mixing zone in the receiving waters, thus incorporating both time of exposure and magnitude for a more accurate assessment compared to existing continuous exposure methods.

Handy (1994) suggested the following three general exposure profiles for pulsed exposure experiments: square, sinusoidal, skewed. Square exposure profiles are the most prevalent in the pulsed literature (Diamond et al. 2006b, Hoang et al. 2007a, Rosen et al. in press). Following a square profile, organisms are transferred directly and immediately between contaminated and clean water. Sinusoidal exposures (McCahon et al. 1991, Fisher et al. 1994, Milne et al. 2000) are similar to square exposures, except these methods incorporate a gradual increase or decrease of the target chemical or sample instead of imposing an abrupt change. Finally, skewed exposures (Widianarko et al. 2001) consist of slowly tapering down from the target concentration, generally by slowly diluting the test solution. Skewed exposures may most closely resemble the dilution of contaminants in receiving environments (Widianarko et al. 2001).

Pulsed exposures have many factors that can make their effects on organisms more complex than standard, continuous exposures (Handy 1994, Reinert et al. 2002, Diamond et al. 2006b, Gordon et al. 2012, Gosset et al. 2016):

1. Pulse magnitude and duration
2. Pulse frequency and recovery
3. Latent effects
4. Test organism age

3.

The following sections (3.13.1 through 3.63.6) summarize the results of published studies that have evaluated the effects of these four factors on toxicity. The knowledge gained from this review is important to support any new more appropriate proposed methodology with scientific rationale and support.

3.1. Pulse Concentration, Duration and Magnitude

In most cases, as the concentration of the contaminant and the duration of exposure increases, toxic effects also tend to increase (Reinert et al. 2002, Diamond et al. 2006a, Stransky et al. 2015, Gosset et al. 2016, Rosen et al. in press). A summary of selected pulsed exposure tests exploring the

relationship between toxicity and pulse duration and concentration is provided in Table 3-1~~Table 3-1~~.

There is a general hypothesis that a chemical must concentrate in the organism and reach a critical burden before effects are observed (Mancini 1983, Diamond et al. 2006b). This means that at short durations, even exceptionally high toxicant concentrations may not show effects if this critical burden is not met. How long it takes the toxicant to reach this critical burden depends on several factors, including mechanism of uptake/depuration, detoxification, and toxicity of a particular toxicant in a particular organism (Mancini 1983, Butcher et al. 2006). For example, copper uptake in the marine diatom *Phaeodactylum tricornutum* was higher in longer pulses at lower concentrations then short pulses at higher concentrations (Angel et al. 2015). The authors suggested this was due to the saturation of algal membrane transport proteins at the higher concentrations (Angel et al. 2015).

Hoang et al. (2007a) performed studies on four metals (zinc, copper, selenium, arsenic) using the standard 21-d protocol for *Daphnia magna* to determine if pulse duration or pulse magnitude was more important to predicting toxicity. For each pair of toxicity tests, when the concentration was doubled, the duration was halved, resulting in equal AUC (area under the curve; concentration × duration) metal exposures. From the data shown in Figure 3-1~~Figure 3-1~~, it is apparent different metals exhibit varying levels of effect dependent on both magnitude and duration of exposure. For example, greater pulse concentrations had a larger effect than longer pulse durations for Cu and As, while the opposite was true for zinc where longer durations appeared to have a larger effect than higher concentrations.

Though results vary depending on the specific species, chemical, and experimental design, a predominant observation among available studies reviewed is that most studies found that organisms have an assimilative ability to tolerate short-term exposures at elevated concentrations better than longer term continuous exposures of the same concentration, and lower equivalent time-weighted concentrations in many cases as well. Furthermore, any effects due to short-term pulses, whether more tolerable or not, can be captured by having an appropriate post exposure observation period as discussed further in Section 3.3~~3.3~~.

Commented [HT3]: The same or lower? For long term exposure with lower concentrations, organisms can develop defense mechanism such as metallothionein or granules that allow the organism to tolerate better also

Stormwater Testing Examples

Stormwater is a complex mixture of contaminants (Burton et al. 2000) and is an episodic discharge in highly urbanized environments. Using a laboratory pulsed experiment, *Strongylocentrotus purpuratus* and *Americamysis bahia*, Rosen et al. (in press) tested six stormwater samples from Naval Base San Diego using standard, continuous exposures (EPA 1995) and modified pulse exposures with pulses of 3-12 h at test initiation for *A. bahia* and just 12 h for *S. purpuratus*. These pulses were determined through an analysis of rainfall duration in San Diego, as rainfall and discharge duration of has been shown to be similar at Naval Base San Diego (Katz et al. 2006). For the pulsed treatments of 3 and 6 h for *A. bahia* and 12 h for *S. purpuratus*, the pulsed procedure demonstrated toxicity in fewer of the samples than the standard, continuous method. This suggests

that the WET methodologies may overestimate toxicity of stormwater discharges in highly urbanized environments (Rosen et al. in press).

Using a different approach to test stormwater using pulsed exposures, Dupuis and Kreutzberger (2003) exposed *Ceriodaphnia dubia* and *Mysidopsis bahia* to sequential, discrete samples during a storm event from two sites (one freshwater, one saltwater). The discrete samples during the duration of the storm were not composited; instead, the animals were transferred sequentially from sample to sample, after the appropriate duration time, for a testing period equal to length of the storm. After these procedures, animals were transferred to clean water for the remainder of the 2-d acute toxicity and 7-d chronic toxicity bioassay. The animals subjected to these treatments demonstrated no, or weak, toxic effects, whereas animals exposed continuously following WET methods found significant toxicity for the samples and chemical analyses found substantial exceedances of water quality standards. These results suggest, once again, that traditional exposure durations based on continuous exposures are not good predicators of toxicity for wet-weather events (Dupuis and Kreutzberger 2003).

It is worth noting that the standard acute methods by EPA already do recognize the need for varying exposure regimes depending on site-specific conditions given the published options for 24, 48, or 96-hour exposures in the manuals. These options are up for consideration as a proposed method for pulsed exposures is developed through this program.

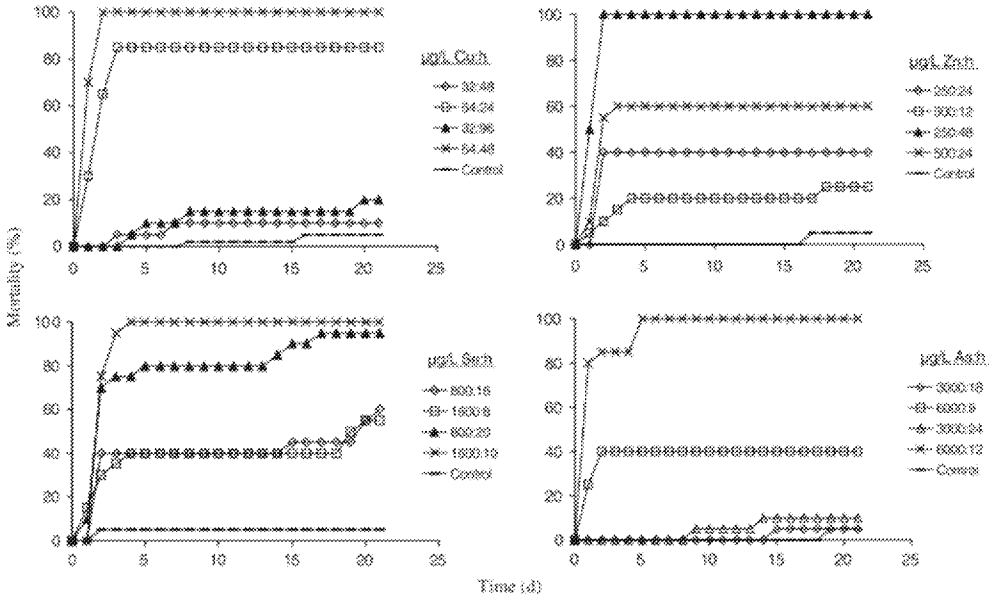

Figure 3-1. Data showing the relative effect of concentration and pulse duration on survival of *D. magna*. Data are averages of all replicates (n=4). (From Hoang et al. 2007a.)

Table 3-1. Selected Studies for Pulsed Duration and Chemical Concentration

Chemical	Species	Pulse Duration	Chemical Conc.	End Point	Conclusions	Study
Arsenic	*Daphnia magna*	9-24 h	3000-6000 µg/L	Survival	For single pulses, the metal concentration had a stronger effect than pulse duration on survival	Hoang et al. 2007c
Bifenthrin	*Holmesimysis costata*	0.5-3 h	25-500 ng/L	Survival	Longer pulses led to lower LC50 values (i.e. more sensitivity). Results evaluated at both 48 h and 96 h. 48 h LC50s were: >500 ng/L for 0.5, 1, 2 h exposures; and 382 ng/L for a 3 h exposure. Compared to a 48-hr static LC50 = 46 ng/L. 96 h LC50s were >500 for 0.5, and 1 h exposures, 500 ng/L for the 2 h exposure, and 382 ng/L for a 3 h exposure. Compared to a 96-hr static LC50 = 17 ng/L.	Stransky et al. 2015
Chlorine	*Menidia beryllina*	2-8 h	36-546 µg/L	Survival	No significant differences related to pulse duration, but a trend towards longer pulses being more toxic. Toxicity reduced by 1.7-1.8x compared to continuous exposures.	Fisher et al. 1994
			8-181 µg/L	Growth	No difference in sensitivity between pulse durations. All were less toxic by a factor of 2.1 to 4.4x among the various exposure periods compared to continuous exposures	
	Mysidopsis bahia	2-8 h	37-548 µg/L	Survival	No significant differences related to pulse duration, but a trend towards longer pulses being more toxic. Toxicity reduced by 3.0-3.7x compared to continuous exposures.	
			8-169 µg/L	Growth	Shorter pulses showed less sensitivity. All were less toxic by a factor of 2.2 to 4.4x compared to continuous exposures	
				Reproduction	No difference in sensitivity among the different pulse durations. All were less toxic by a factor of 2.2 compared to continuous exposures. Reproduction was the most sensitive endpoint	

Chemical	Species	Duration	Chemical Conc.	End Point	Conclusions	Study
Chlorpyrifos	*Daphnia magna*	1-24 h	0.12-0.5 µg/L	Survival	All daphnids exposed to chlorpyrifos at 0.5 µg/L for ≥12 h had similar survival curves as that observed using continuous exposures. Pulsed treatments at lower concentrations did not elicit a significant effect, but significant reductions in survival were observed in all continuous treatments at each concentration	Naddy et al. 2000
				Growth	No effects on growth were detected for pulsed exposures. There was no survival in the continuous exposure treatments, so growth endpoints were not available for this exposure regime.	
				Reproduction	No effects on reproduction were detected for pulsed exposures. Due to no survival in continuous exposure treatments, no comparison were made to pulsed exposure treatments	
Magnesium	*Americanna cumingi*	4-24 h	0-4000 mg/L	Reproduction	Sensitivity increased non-linearly with pulse duration	Hogan et al. 2013
	Chorella sp.	4-24 h	0-12000 mg/L	Growth	As pulsed duration increased, sensitivity increased linearly	
	Hydra viridissima	4-24 h	0-1500 mg/L	Growth	Sensitivity increased non-linearly with pulse duration	Hogan et al. 2013
	Lemna aequnoctialis	4-24 h	0-4000 mg/L	Growth		
	Moinodaphnia macleayi	4-24 h	0-4000 mg/L	Reproduction		
	Morgunda mogurnda	4-24 h	0-4000 mg/L	Survival		
Selenium	*Daphnia magna*	8-20 h	800-1600 µg/L	Survival	For single pulses, when the time-averaged concentration is the held constant, metal concentration and pulse duration manipulations had similar effects.	Hoang et al. 2007a

Chemical	Species	Duration	Chemical Conc.	End Point	Conclusions	Study
Zinc	*Americamysis bahia*	3-12 h	0.398-16.7mg/L	Survival	Short-term exposures were less toxic than continuous. LC50 values for the 3, 6, 12 h pulses were 10.4, 3.9, and 2.1 mg/L, respectively. These are factors of 11, 6, and 2 greater than that obtained for 96-hr continuous exposures.	Rosen et al. in press
	Daphnia magna	12-48 h	250-500 µg/L	Survival	For single pulses, the metal concentration had a weaker effect than pulse duration on survival.	Hoang et al. 2007a
Copper	*Daphnia magna*	24-96 h	32-64 µg/L	Survival	For single pulses, the metal concentration had a stronger effect than pulse duration on survival.	Hoang et al. 2007a
	Americamysis bahia	3-12 h	88.8-2970 µg/L	Survival	Short-term exposures were less toxic than continuous. LC50 for the 3, 6, 12 h pulses were 1475, 795, and 240 µg/L respectively. These are factors of 11, 6, and 2 greater (i.e. less sensitive) than 96-h continuous exposure values.	Rosen et al. in press
	Holmesimysis costata	0.5-3 h	20-250 µg/L	Survival	Longer pulses led to lower LC50s (i.e. more sensitivity). Results evaluated at both 48 h and 96-h total duration. 48 h LC50s: >250 µg/L for 0.5 and1 h pulses; 131 µg/L for a 2 h pulse, and 93.3 µg/L for a 3 h pulse. Compared to a 48-hr static LC50 of 25.3 µg/L. 96 h LC50s: >250 µg/L for 0.5 and 1 h pulses; 93 µg/L for a 2 h pulse; and 57.9 µg/L for a 3 h pulse. Compared to a 96-hr static LC50 of 20.5 µg/L	Stransky et al. 2015
	Mytilus galloprovincialis	0.5-3 h	20-250 µg/L	Embryo-larval Development	Longer pulses led to lower 48-hr EC50s (i.e. more sensitivity). EC50 >250 µg/L for a 0.5 h pulse; and 189, 131, and 85 µg/L for 1 h, 2 h, and 3 h pulses, respectively. 48-hr static EC50 = 8.9 µg/L.	
	Phaeodactylum tricornutum	0.5-4 h	30-600 µg/L	Growth	Significant interaction between pulse and duration. As either increased, so did toxicity. Using time-averaged concentrations, (TACs) accurately predicted toxicity. Copper uptake was higher in longer pulses at lower concentrations then short at higher concentrations (even with equivalent TACs) possibly because of the saturation of algal membrane transport proteins at the higher concentrations used.	Angel et al. 2015

Chemical	Species	Duration	Chemical Conc.	End Point	Conclusions	Study
Copper	*Strongylocentrotus purpuratus*	3-12 h	15-367 µg/L	Embryo-larval Development	Short-term exposures were less toxic than continuous. EC50 for the 3, 6, 12 h pulses were 296, 223, and 114 µg/L respectively. These are factors of 20, 15, and 8 greater than that for 96-h continuous exposures.	Rosen et al. in press
		0.5-3 h	20-250 µg/L	Embryo-larval Development	Longer pulses led to lower EC50s (i.e. more sensitivity). EC50 >250 µg/L for 0.5, 1, 2 h pulses, and 165 µg/L for a 3 h pulse. 96-hr static EC50 = 14.9 µg/L.	Stransky et al. 2015

3.2. Pulse Exposure Frequency

Pulse frequency is another important factor that may affect the toxicological outcome of intermittent exposures. Selected pulsed exposure tests exploring the effects of recovery periods between pulse exposures are presented in Table 3-2~~Table 3-2~~. Most importantly, infrequent pulses have a period between them that allow organisms to recover from the first pulse. The benefit of a recovery period has been shown both with metal (Pynnönen 1990, Bearr et al. 2006, Diamond et al. 2006b, Hoang et al. 2007a, 2007c) and organic contaminants (Kallander et al. 1997, Naddy and Klaine 2001, Zhao and Newman 2006). In general, the longer the pulse, and the higher the concentration, the longer a recovery period is needed for the organisms to return to pre-pulse condition (Hoang et al. 2007b). However, there are examples of animals, such as fish, acclimating to exposure relatively rapidly using physiological products (such as the protein metallothionein) to tolerate contaminant exposure temporarily. The length these adaptions stay in place is related to the energy it takes to maintain them (Laurén and McDonald 1987, Bearr et al. 2006, Diamond et al. 2006b).

Diamond et al. (2006b) studied the effects of time-varying exposures of copper, zinc, and ammonia on *Daphnia magna* and *Pimephales promelas*. Both species demonstrated effects of a second pulsed exposure for copper and zinc, but the effect was different for each species. Using copper as an example (the effect was similar for zinc), *D. magna* showed increased survival with increased recovery times between two 24 h pulses of 32 µg/L copper (Figure 3-2~~Figure 3-2~~). In contrast, *P. promelas* showed decreased survival if the two 24 h pulses of 40 µg/L copper were further apart (96 or 120h), compared to pulses that were no more than 48h apart (Figure 3-2~~Figure 3-2~~). This difference is likely due to the different mechanisms the organisms use to tolerate copper. *D. magna* is not as easily able to regulate its internal copper concentration, so the closer two pulses are together, the more likely it will increase beyond its critical burden, resulting in mortality (Mancini 1983, Diamond et al. 2006b). This has been shown in *D. magna* with copper, as well as zinc, selenium, and arsenic (Hoang et al. 2007a) and copper and phenol with *Hyalella azteca* (Zhao and Newman 2006). *P. promelas* regulates copper with metallothionein, which is both time-dependent for how quickly it engages, and how long it remains protective. Previous research suggests that for *P. promelas* this window is 48-96 h, in agreement with the observation described above (Bearr et al. 2006, Diamond et al. 2006b, Zahner et al. 2006).

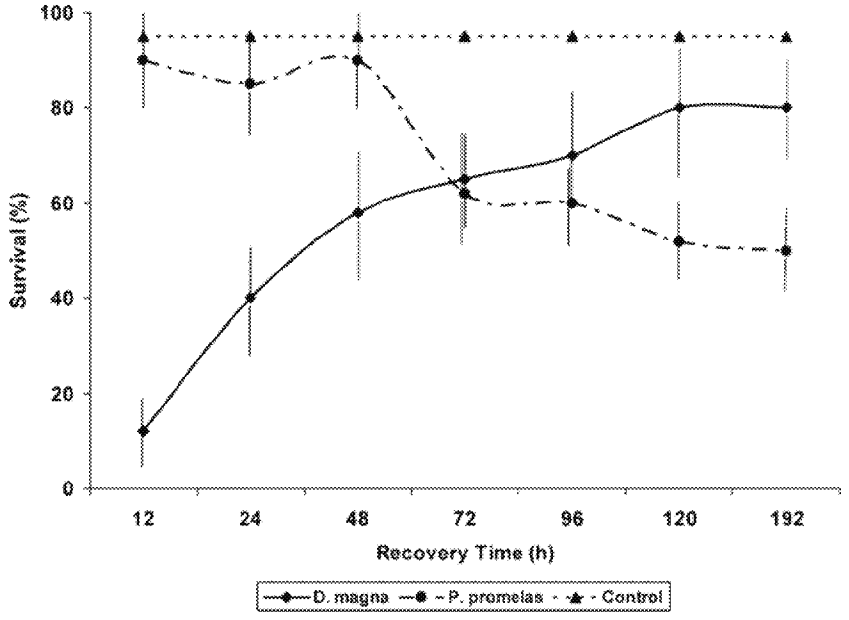

Figure 3-2. The effect of recovery time on the survival of *D. magna* and *P. promelas* (Diamond et al. 2006).

A few studies where the time-averaged concentration (TAC = concentration × duration) is the same between exposure profiles (fluctuating recovery, concentration, and pulse durations) have found no effect (including fluctuating recovery periods) on the survival of exposed organisms (Angel et al. 2010, 2015, 2017). This makes sense for species that are similar to *D. magna* in the example above; if the recovery period prevents the organism from hitting the critical toxicant burden, several pulses at lower concentrations or fewer pulses at higher concentrations should have somewhat similar effects. However, for circumstances, such as *P. promelas* above, were the organism has a temporary acclimation to the contaminant, this relationship will likely be more complicated.

In summary, multiple pulses have been shown to exhibit a variety of different effects depending on a number of different design factors and species/endpoint selection. It is good to be aware of these factors and complexities, but from a practicality standpoint the implementation of multiple exposure test regimes may be impractical for routine compliance testing. Instead, a conservative pulse duration that captures the period of time where multiple pulses may occur is recommended.

Table 3-2. Selected Studies for Effects of Recovery Periods Between Pulses

Chemical	Species	Pulsed Duration	Chemical Conc.	No. Pulses	Recovery Duration	End Point	Effect?	Description	Study
Aldicarb	*Chironomus riparius*	1-2 h	26 µg/L	2	0-24 h	Mobility	Yes	1 h pulses were less toxic than 2 h pulses as long as there was at least 2-6 h between pulses. Controlled so the concentration was the same for each treatment.	Kallander et al. 1997
Arsenic	*Daphnia magna*	6 h	4000-6000 µg/L	2	24-168 h	Survival	Yes	After a recovery time of less than 96 h, mortality due to the first pulse was greater than mortality due to a second pulse. At 96 h, the mortality was the same in both pulses, or slightly lower in the second pulse	Hoang et al. 2007a
						Growth	No	No differences in growth among the different pulsed exposure treatments compared to controls	
						Reproduction	No	No differences in reproduction among the different pulsed exposure treatments to controls	
		3-120 h	3000-6000 µg/L	2	0-168 h	Survival	Yes	Increased recovery time led to increased mortality except at the highest concentration 6000 µg/L where there was no effect	Hoang et al. 2007c
						Growth	No	No effects of arsenic exposure on growth compared to controls	
						Reproduction	No	With recovery periods of 24 and 96 h, the time to first brood was longer than for controls	
Cadmium	*Pimephales promelas*	6-12 h	40-60 µg/L	2	≥24 h	Survival	No	No changes in survival during a recovery period	Diamond et al. 2005

Chemical	Species	Pulsed Duration	Chemical Conc.	No. Pulses	Recovery Duration	End Point	Effect?	Description	Study
Carbaryl	*Chironomus riparius*	1-2 h	40 µg/L	2	0-24 h	Mobility	Yes	1 h pulses were less toxic than 2 h pulses as long as there was at least 2-6 hours between pulses. Controlled so the concentration was the same for each treatment	Kallander et al. 1997
Carbofuran	*Chironomus riparius*	1-2 h	2.5 µg/L	2	0-24 h	Mobility	Yes		
Chlorpyrifos	*Daphnia magna*	1.5-12 h	0.5-1 µg/L	2	3-12 h	Survival	Yes	More than 72 h was needed for a full recovery from exposure to chlorpyrifos. In some cases, when a critical exposure threshold was met, there was no recovery	Naddy and Klaine 2001
						Immobility	Yes		
						Reproduction	No	Recovery time had no effect on responses except when survival was impacted	
						Growth	No		
Copper	*Daphnia magna*	12-24 h	8-48 µg/L	2	12-480 h	Survival	Yes	Increased recovery time between pulses led to increased survival	Diamond et al. 2006
						Reproduction	Yes	After a few days of recovery, reproduction increased to, or exceeded, reproduction in the controls	
		12 h	32-64	2	24-192 h	Survival	Yes	Pulses acted independently with a recovery period of at least 96 h. A recovery period of 48 h or less resulted in a larger effect for the second pulse.	Hoang et al. 2007a
						Growth	No	No differences in growth among the different pulsed exposure treatments compared to controls	
						Reproduction	No	No differences in reproduction among the different pulsed exposure treatments to controls	

Chemical	Species	Pulsed Duration	Chemical Conc.	No. Pulses	Recovery Duration	End Point	Effect?	Description	Study
Copper	*Hyalella azteca*	12 h	0.8-1.1 mg/L	2	0-72 h	Survival	Yes	Amphipods were more sensitive to a second pulse of copper unless recovery time was sufficiently long enough to return to an initial, resistant state (~83 h)	Zhao and Newman 2006
	Melita plumulosa	8-96 h	100-900 µg/L	2-3	24-144 h	Survival	No	When the time-averaged-concentrations (TAC) remained consistent, there was no effect of recovery time	Angel et al. 2010
	Pimephales promelas	3-24 h	5-30 µg/L	2	0-144 h	Survival	Yes	A 48-h recovery time resulted in significantly greater fish survival than that observed with either shorter or longer recovery times; likely due to the induction and effectiveness time of metallothionein	Bearr et al. 2006
			40 µg/L	2	0-144 h	Growth	No	No effects on growth/biomass in pulsed 40 µg/L treatments over 14d test periods	
		24 h	30-40 µg/L	2-3	48-120 h	Survival	Yes	Adaptation to pulsed copper activated for approximately 48-96 h. After 96 h the fish is just as susceptible to pulses of copper	Diamond et al. 2006
	Phaeodactylum tricornutum	0-8 h	30-600 µg/L	2-3	16-48 h	Growth	No	When TAC remained the same, there was no effect of recovery time. After exposures, growth increased temporarily	Angel et al. 2015
Dimethoate	*Daphnia magna*	0.5-6 h	10-20 mg/L	1-2	48 h	Immobility	Yes	Some mobility recovered following first pulse once placed in clean media. Increased sensitivity observed for second pulse after 48 h recovery	Andersen et al. 2006
Malathion	*Chironomus riparius*	1-2 h	32 µg/L	2	0-24 h	Mobility	No	Two 1 h pulses were equally toxic as one two-hour pulse, regardless of recovery period. The authors suggest this is due to lack of reactivation of AcHE after exposure. Concentrations did not change by regime	Kallander et al. 1997
Parathion	*Chironomus riparius*	1-2 h	55 µg/L	2	0-24 h	Mobility	No		

Chemical	Species	Pulsed Duration	Chemical Conc.	No. Pulses	Recovery Duration	End Point	Effect?	Description	Study
Primicarb	*Daphnia magna*	0.5-6 h	40-70 mg/L	1-2	48 h	Immobility	Yes	Some mobility recovered following first pulse once placed in clean media. Increased sensitivity observed for second pulse after 48 h recovery	Andersen et al. 2006
Propoxar	*Chironomus riparius*	1-2 h	25 µg/L	2	0-24 h	Mobility	Yes	1 h pulses were less toxic than 2 h pulses as long as long as there was at least 2-6 h between pulses. These tests were controlled so the dose was the same	Kallander et al. 1997
Selenium	*Daphnia magna*	8 h	800-1600 µg/L	2	72-288 h	Survival	Yes	Separating pulses by more than 72 h resulted in less toxicity, suggesting that the organism could develop tolerance	Hoang et al. 2007a
						Growth	No	No differences in growth among the different pulsed exposure treatments compared to controls	
						Reproduction	No	No differences in reproduction among the different pulsed exposure treatments to controls	
		3-12 h	800-1800 µg/L	2	0-288 h	Survival	Yes	Pulses without recovery time resulted in lower survival than treatments with recovery time. In addition, mortality was higher during the first pulse than the second pulse, except at the highest concentration	Hoang and Klaine 2008
						Growth	No	No differences in growth among the different pulsed exposure treatments compared to controls	
						Reproduction	No	No differences in reproduction among the different pulsed exposure treatments to controls	

Chemical	Species	Pulsed Duration	Chemical Conc.	No. Pulses	Recovery Duration	End Point	Effect?	Description	Study
Zinc	*Daphnia magna*	3-24 h	250-2500 µg/L	2	12-480 h	Survival	Yes	Increased recovery time between pulses led to increased survival	Diamond et al. 2006
						Reproduction	Yes	After a few days of recovery following a single pulse, reproduction met or exceeded that in the controls	
		24 h	250-500 µg/L	2	24-168 h	Survival	Yes	Responses to pulses acted independently when there was a recovery period of at least 96 h. A recovery period of less than 96 h resulted in a greater effect following a second pulse	Hoang et al. 2007a
						Growth	Yes	No effect of recovery time on growth except at 375 µg/L with a 96 h recovery time and 500 µg/L with a 168 h recovery, both which caused a significant decrease in growth relative to controls	
						Reproduction	Yes	No effect of recovery time except at 375 µg/L with a 96 h recovery time where reproduction was significantly lower relative to that observed in the controls	
	Pimephales promelas	24 h	300-400 µg/L	2-3	48-120 h	Survival	Yes	A longer recovery time resulted in significantly greater fish mortality (i.e. mortality continued after the pulse)	Diamond et al. 2006

3.3. Latent Effects

A growing body of research demonstrates that post-exposure effects are important to consider when evaluating effects due to pulsed exposures. For acute exposures, researchers have found latent mortality in several species, including fish and several crustacean groups, that can be delayed up to several days, sometimes weeks (Brent and Herricks 1998, Reinert et al. 2002, Diamond et al. 2006b, Angel et al. 2010, Gordon et al. 2012). This suggests that toxicity testing that does not incorporate a post-exposure observation period may underestimate potential for effects. It suggests that short exposures with high toxicant concentration could be as toxic, or more toxic, than longer exposures with lower toxicant concentrations (Burton et al. 2000).

For chronic endpoints, such as growth and reproduction, a post-exposure observational period can demonstrate recovery of the test organisms from pulsed exposures. For example, freshwater and marine algae have shown recovery of growth at faster rates than control treatments following an episodic exposure to metals (Hogan et al. 2013, Angel et al. 2015, 2017) and *D. magna* displayed higher growth rate and increased fecundity, compared to controls, after exposure to copper (Diamond et al. 2006b), possibly due to a hormetic effect. This suggests that exposed organisms in some cases may "catch up" to the performance of control organisms during the post-exposure period, resulting in no difference between control and pulsed organisms for these endpoints at the end of the test (Diamond et al. 2006b).

Latent, or delayed, effects from toxicant exposure have been demonstrated several times in the pulsed exposure literature, for metals (Abel and Garner 1986, Brent and Herricks 1998, Diamond et al. 2006b, Zhao and Newman 2006, Hoang et al. 2007a, 2007c, Hoang and Klaine 2008, Angel et al. 2010) and organic (Abel and Garner 1986, Naddy and Klaine 2001) contaminants. In general, failure to account for latent mortality can result in underestimating toxicity for effluent discharges, especially with current WET methodologies that do not currently have a post-exposure observation time (Brent and Herricks 1998, Burton et al. 2000, Diamond et al. 2006b). Understanding the mechanism of uptake and toxicity of a contaminant will help determine if it will demonstrate latent effects; if mechanism is unknown, it is safer to assume latency (Reinert et al. 2002).

Brent and Herricks (1998) evaluated the post-exposure effects of cadmium, zinc, and phenol for three standard toxicity testing organisms; *Ceriodaphnia dubia*, *Hyalella azteca*, and *Pimephales promelas*. They found post-exposure immobility (as a proxy for mortality) to be a good measure of effects following exposure to cadmium and zinc. In general, they found that the time to see an effect was shorter when pulses were more frequent in duration and greater in concentration (i.e. higher dose of contaminant), meaning that higher does led to faster, greater effects (Figure 3-3~~Figure 3-3~~). In addition, they found that maximum effect times for *C. dubia* (mean = 44.1 h) were significantly shorter than for either *H. azteca* or *P. promelas* (mean = 89.3 h and 103.0 h, respectively). On the other hand, phenol exposure caused no effect for *H. azteca* and immobility of *C. dubia* was more pronounced immediately after the pulse with recovery (returned mobility) noted during the post-exposure period (Brent and Herricks 1998). It appears that

requisite post-exposure observation period lengths depend on the species and toxicity of the sample (Brent and Herricks 1998, 1999).

Based on the observations noted here we propose to include a post-exposure period as a part of a standard methodology where the entire exposure period (pulse plus post observation in lab control or receiving water) will be equivalent to that used for the current standard WET methods.

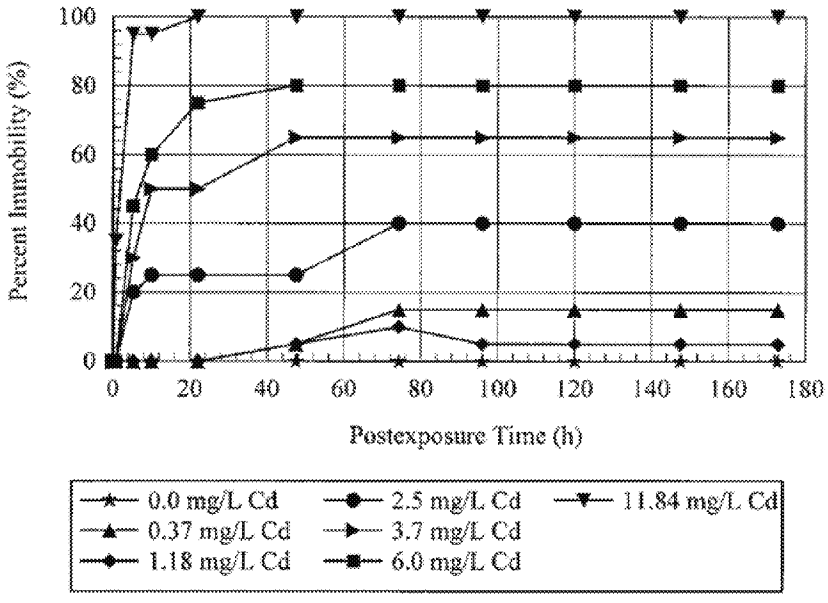

Figure 3-3. Time to mortality for *C. dubia* following exposure to different concentrations of cadmium. The origin of the graph represents the end of the 2 h pulse of Cd at the indicated concentration. Maximum effect indicated by flat lines. (From Brent and Herricks 1999).

This figure shows varying degrees of daphnia mobility up to an approximate 80-hour post exposure period depending on the test concentration.

Table 3-3. Selected Studies on Latency Effects.

Chemical	Species	Pulse Duration	Chemical Conc.	Post-Exposure Duration	Endpoint	Effect ?	Description	Study
Arsenic	*Daphnia magna*	3-120 h	3000-6000 µg/L	13-20 d	Survival	Yes	Latent mortality was indicated in all treatments	Hoang et al. 2007c
Cadmium	*Ceriodaphnia dubia*	15-240 m	0.37-11.84 mg/L	6-172 h	Immobility	Yes	Latent effects were observed during post exposure period with no recovery. Higher doses resulted in greater effects occurring sooner. At highest Cd concentration, exposure for 30 m was sufficient to cause 100% immobility	Brent and Herricks 1998
	Hyalella azteca	15-240 m	0.06-0.019 mg/L	6-144 h	Immobility	Yes	Latent effects were observed during post exposure period with no recovery. Higher doses resulted in greater effects occurring sooner. At highest Cd concentration, exposure for 30 m was sufficient to cause 95% immobility	
	Pimephales promelas	15-240 m	0.4-12.8 mg/L	6-168 h	Immobility	Yes	Latent effects were observed during post exposure period with no recovery. Higher doses resulted in greater effects occurring sooner. At highest Cd concentration, exposure for 30 m was sufficient to cause 85% immobility	
	Gammarus pulex	1-200 m	0.5-5 mg/L	14 d	Survival	Yes	Mortality continued to occur throughout the 14 d exposure period after the short exposures	Abel and Garner 1986
	Pimephales promelas	6-12 h	40-60 µg/L	60-138 h	Survival	No	No latency effects (i.e. no additional mortality after exposure ended)	Diamond et al. 2005
					Biomass	No	No effect on biomass due to Cd exposure observed (no latency effect either)	
Chlorpyrifos	*Daphnia magna*	1.5-12 h	0.12-1.0 µg/L	1-72 h	Survival	No	No latency effects (i.e. no additional mortality after exposure ended)	Naddy and Klaine 2001
					Reproduction	No	No effect on reproduction due to chlorpyrifos exposure observed (no latency effect either)	
					Growth	No	No effect on growth due to chlorpyrifos exposure observed (no latency effect either)	

Chemical	Species	Pulse Duration	Chemical Conc.	Post-Exposure Duration	Endpoint	Effect?	Description	Study
Copper	*Melita plumulosa*	4-160 h	100-900 µg/L	24-240 h	Survival	Yes	Negligible mortality was observed during the exposures. Latent mortality was observed in the subsequent 96 h non-exposure period, after which negligible additional mortality occurred during the remainder of the 240 h tests. Latent mortality exhibited a strong relationship with the time-averaged concentration	Angel et al. 2010
	Pimephales promelas	6-12 h	50-75 µg/L	60-138 h	Survival	No	No latency effects (i.e. no additional mortality after exposure ended)	Diamond et al. 2005
					Biomass	No	No effect on biomass due to Cu exposure observed (no latency effect either)	
	Hyalella azteca	12 h	0.8-1.1 mg/L	70-80 h	Survival	Yes	Latent mortality observed between 60-70 h post exposures	Zhao and Newman 2006
Selenium	*Daphnia magna*	4-25 h	800-2000 µg/L	~20 d	Immobility	Yes	No mortality during initial exposure, but increasing latent mortality with increasing concentration and/or duration	Hoang and Klaine 2008
Zinc	*Ceriodaphnia dubia*	15-240 m	0.15-4.8 mg/L	6-144 h	Immobility	Yes	Latent effects were observed during post exposure period with no recovery. Higher doses resulted in greater effects occurring sooner. At the highest Zn concentration, exposure for 30 m was sufficient to cause 100% immobility	Brent and Herricks 1998
	Daphnia magna	3-24 h	250-2500 µg/L	0-474 h	Survival	Yes	20-60% mortality in 24 h pulsed exposures and continued mortality effects in post exposure up to 96-120 h	Diamond et al. 2006
		12 h	750 µg/L	20.5 d	Survival	Yes	Latent mortality increased as a function of concentration and pulse duration	
	Hyalella azteca	15-240 m	2-64 mg/L	6-144 h	Survival	Yes	Latent effects were observed during post exposure period with no recovery. Higher doses resulted in greater effects occurring sooner. At highest Zn concentration, exposure for 30 min was sufficient to cause 30% immobility	Brent and Herricks 1998
	Pimephales promelas	24h	300-400 µg/L	0-72 h	Survival	Yes	20-60% of mortality during 24 h pulse, with continued mortality for 4 d post pulsed exposure	Diamond et al. 2006

3.4. Organism Age at Initiation

There is a general assumption in toxicology that young organisms are more sensitive to toxicant exposure than older organisms. While this has held true in several studies that have looked at the effect of age on toxic effects after pulsed exposures (Holdway and Dixon 1985, Williams and Holdway 2000, Andersen et al. 2006), there are examples when the relationship between age and sensitivity is not so clear (Holdway and Dixon 1988, Hoang and Klaine 2007). Holdway and Dixon, using permethrin, tested if larval *Catostomus commersoni* (white suckerfish) and juvenile *Jordanella florida* (flagfish) susceptibility to brief exposures of permethrin were modified by age. *C. commersoni* was more sensitive to permethrin at 20 d post-hatch larvae, compared to 13 and 26 d old larvae. *J. florida* was most sensitive at 8 d compared to 2 and 4 d old fish. The authors offered saltatory ontogeny as a possible explanation; differences in sensitivity depend on the development process, specifically during the targeted tissue/organ's most critical stages of development. Similarly, exposing *D. magna* to four metals (Cu, Zn, Se, As), Hoang and Klaine (2007) found increasing sensitivity as *D. magna* aged to a certain point (4 d for Cu and Zn and 2-3 d for As and Se), and then decreasing sensitivity as the organism age increased (Figure 3-4~~Figure 3-4~~).

The current EPA and ASTM standard methods recognize the impacts that different ages may have on test organism sensitivity and have therefore incorporated restrictions on the ages and/or sizes of test organisms used for all WET testing procedures. Proposed toxicity test methods to evaluate episodic discharges will conform to the age requirements in existing WET protocols since those ages are assumed (or demonstrated to be) most sensitive.

This summary on test organism age is thus included for context only and to also provide references in case pulses might be desired at different times during an exposure period to better mimic or validate specific conditions. For example, when conducting special study *in situ* exposures, there may be some age-related variances from standard protocols based on when a pulse occurs in the environment.

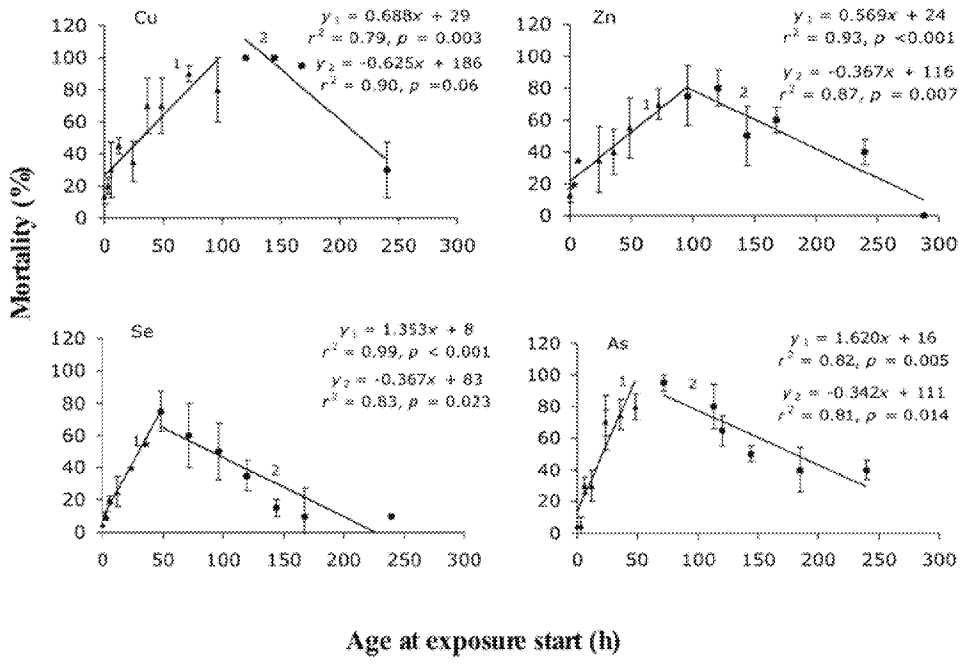

Figure 3-4. Sensitivity of *Daphnia magna* to a 12h dose of Cu, Se, Zn, and As at different ages during the exposure. Cu 70 µg/L; Zn 750 µg/L; Se 1,000 µg/L; As 5,000 µg/L. (From Hoang and Klaine 2007).

Table 3-4. Selected Studies on Age Effects in Pulsed Exposure Studies

Chemical	Species	Age	Pulse Duration	Chemical Conc.	Endpoint	Effect?	Description	Study
Arsenic	*Daphnia magna*	3h-10 d	12 h	1000 µg/L	Survival	Yes	Juveniles were more sensitive to As than adults with a peak sensitivity at 2 days post-hatch	Hoang and Klaine 2007
					Reproduction	Yes	Organisms showed decreased cumulative reproduction with age between 3-72 h old, but increased cumulative reproduction with increasing age between 72-240 h	
Cadmium	*Melanotaenia fluviatilis*	3-92 h embryos 3-10 d larvae	2 h	0.033,0.1,0.33,1,3.3 mg/L	Development	Yes	Pulse-exposed metals at moderate concentrations can significantly affect the early life stages	Williams and Holdway 2000
Copper	*Daphnia magna*	3h-10 d	12 h	70 µg/L	Survival	Yes	Juveniles were more sensitive to Cu than adults with a peak sensitivity of 4 days post-hatch	Hoang and Klaine 2007
				70 µg/L	Reproduction	Yes	Organisms showed decreased cumulative reproduction with age between 3 h-96 h old, but increased cumulative reproduction with increasing age between 96-240 h	
Dimethoate	*Daphnia magna*	≤24hr and 3d	0.5-6 h	10-30 mg/L	Immobility	No	No effect based on age	Andersen et al. 2006

Chemical	Species	Age	Pulse Duration	Chemical Conc.	Endpoint	Effect?	Description	Study
Fenoxycarb	*Daphnia magna*	<24 h, 4-6 d, 8 d, 11 d	Diluted pulse varied with conc.	0.2, 0.8, 3.2, 13, 50 µg/L	Survival	No	There were no significant effects on survival or time to first brood of first- and second generation daphnids in any age group.	Hosmer et al. 1998
					Reproduction	Yes	The number of young per daphnid was significantly lower than control daphinds only among daphnids that were ≤24 h old at test initiation and exposed to the highest initial measured concentration of fenoxycarb (45 µg/L)	
Magnesium	*Moinodaphnia macleayi*	onset of test, onset of reproduction	4-24 h	0-3000 mg/L	Reproduction	Yes	Cladocerans were 2-5x more sensitive when exposed at reproductive maturity, compared to juveniles	Hogan et al. 2013
Methoxychlor	*Jordanella floridae*	2,4,8 d post hatch larvae	2 h	1.29, 2.58, 3.51, 5.48 mg/L	Mortality	Yes	Younger organisms fed during the test were more sensitive to methoxychlor with respective mean LC50 values of 3.2, 13.5 and 38.6 mg/L for 2, 4 and 8 d fish. Conversely there was no impact of age on LC50s (mean values 1.6 - 3.8 mg/L) for fish that were not fed during the exposure period.	Holdway and Dixon 1985
Permethrin	*Catostomus commersoni*	12,30,26 d post hatch larvae	2 h	0.0001, 0.001, 0.01, 0.1 mg/L	Survival	Yes	Sensitivity was dependent on age with younger organisms exhibiting greater	Holdway and Dixon 1988
	Jordanella floridae	2,4,8 d post hatch larvae	2 h	0.2, 0.6, 1.2, 2.0 mg/L	Survival	Yes		

Chemical	Species	Age	Pulse Duration	Chemical Conc.	Endpoint	Effect?	Description	Study
Selenium	*Daphnia magna*	3h-10 d	12 h	5000 µg/L	Survival	Yes	Juveniles were more sensitive to Se than adults with a peak sensitivity at 2 days post-hatch	Hoang and Klaine 2007
					Reproduction	Yes	Organisms showed decreased cumulative reproduction with age during exposures between 3-48 h old, but increased cumulative reproduction with increasing exposure age between 48-240 h	
Zinc	*Daphnia magna*	3h-10 d	12 h	750 µg/L	Survival	Yes	Juveniles were more sensitive to Zn than adults with a peak sensitivity between at 4 days post-hatch	Hoang and Klaine 2007
					Reproduction	Yes	Exposure of young organisms (3-96 h old) resulted in less reproduction than that for organisms exposed at an older age between 96-240 h	
	Melanotaenia fluviatilis	embryos: 3-92h; 3-10 d larvae	2 h	0.33,1,3.3, 10,33.33 mg/L	Development	Yes	For the <24hr, 3-4 d, and 9-10 d, the factor increase in LC50 for the two-hour pulse (compared to a 96 h continuous exposure) were; 1.9, 2.1, and 5.8 respectively (higher values indicate less sensitivity).	Williams and Holdway 2000

3.5. Mixture of Toxicants and Complex Samples

Exposures of mixtures of toxicants to determine joint toxicity effects is becoming increasingly common in the toxicological literature (Bailey et al. 1997, Sharma et al. 1999, Phillips et al. 2003, Verslycke et al. 2003, Diamond et al. 2017, Posthuma et al. 2017, Lynch et al. 2016, Perez and Hoang 2017, 2018), but is still uncommon in the pulsed exposure literature (Brent and Herricks 1999, Dupuis and Kreutzberger 2003, Holth et al. 2008, Kim et al. 2008, Rosenkrantz et al. 2008, de Zwart et al., 2017, Rosen et al. in press). Joint toxicity can result in additive toxicity, or non-additive toxicity (e.g. antagonistic, synergistic). The proposed pulsed exposure and current standard continuous exposure methods both equally address mixtures by testing the whole effluents or receiving waters, highlighting the importance of toxicity testing as a risk assessment and regulatory management tool.

3.6. Selection of Toxicity Testing Endpoints

In continuous exposures, both acute (lethality or immobility) and chronic (sublethal) endpoints are valuable to determine toxicity of samples, with chronic endpoints commonly being more sensitive. However, acute toxicity endpoints are frequently more sensitive to pulsed exposure than chronic endpoints in daphnia and standard fish tests (i.e. many studies find effects of survival with pulsed exposures, usually chronic endpoints seem less affected) (Reinert et al. 2002, Diamond et al. 2006a, Hoang et al. 2007c). These observations may reflect the types of chemicals tested using both acute and chronic endpoints. Theoretically, chemicals that bioaccumulate readily such as selenium or many organic chemicals might show greater sensitivity using chronic endpoints if given a sufficient number of pulses over time. Fast acting chemicals such as copper, chloride, ammonia, and zinc, will have acute endpoints that are more sensitive. Indeed, there have been studies that have found chronic effects after pulsed exposures (Fisher et al. 1994, Williams and Holdway 2000, Hoang and Klaine 2007, Stransky et al. 2015, Rosen et al. in press). In general, more sensitive chronic endpoints have been reproduction (Fisher et al. 1994, Hoang and Klaine 2007) or development (Williams and Holdway 2000, Rosen et al. in press), whereas growth has been less reliable. One possible reason growth has been a less reliable endpoint is because many organisms have demonstrated faster growth immediately after a pulsed exposure, appearing to "catch up" to the control organisms, potentially making it difficult to see differences at the end of the test (Diamond et al. 2006b, Hogan et al. 2013, Angel et al. 2015, 2017).

In conclusion the endpoints selected should cater to and appropriately reflect site-specific exposure potential. For example, an acute lethality endpoint may be most appropriate for exposure to a very transient undiluted end-of-pipe sample, whereas chronic endpoints of growth and survival are more applicable for the mixing zone of the receiving waters where longer-term exposures might be expected.

4. Alternative and Supplementary Approaches to Evaluating Episodic Exposures

4.

4.1. Dilution/Mixing Zones

Incorporating an appropriate dilution concentration that may be tested based on site-specific mixing zone characteristics is a primary identified alternative approach for testing samples that come from episodic events. Extensive mixing zone studies in the receiving environment are required to come up with an appropriate dilution credit that can be applied to end-of-pipe measurements/results from NPDES monitored discharges. Determination of appropriate mixing zones can be conducted through a number of different means. Two such methods are the USEPA-approved steady-state model Cornell Mixing Zone Expert System (CORMIX) or dye-studies. These methods can be used either alone or in conjunction depending on site specific requirements to develop representative conservative dilution factors within zones of initial dilution that may be incorporated into permit calculations (Early et al. 2007).

However, mixing zones or dilution credits may not always be authorized by water quality regulators as is the current case for Naval Base San Diego. Currently the San Diego Regional Water Quality Control Board does not authorize mixing zones or dilution credits in water bodies that have current water quality impairment listings as is the case for San Diego Bay.

Commented [LJ4]: Neither is Pulsed exposures. So eliminating this alternative for this justification is not valid. I recommend this be included in the demonstration plan as a side be side comparison with pulsed exposures using a 24 hr composite approach.

While other locations may allow for dilution credits, modelling exposure with very conservative assumptions, or potential costly field studies, must still be conducted on a site-specific basis to determine appropriate allowable mixing zones. Furthermore, applying a dilution to a test sample addresses magnitude of exposure, but fails to account for time of exposure; both of which are important factors with regard to toxicological effects and protection of aquatic life.

Ideally, a more realistic approach will incorporate both time and magnitude as a part of an exposure regime as addressed through the literature review presented herein.

Commented [LJ5]: This should be tested not assumed.

4.2. Toxicity Modelling

Toxicological modeling techniques have also been applied in some circumstances to predict toxicity due to intermittent discharges to organisms in the receiving environment. These models, while improving, still have too much uncertainty and are too context dependent to realistically be available for regulatory use (Diamond et al. 2006a, Gordon et al. 2012, Gosset et al. 2016). This will require additional inputs from quality ecotoxicological studies to hopefully minimize this uncertainty, which led Gordon et al. (2012) to create a database of pulsed exposure studies. Once again, a detailed review of these models is beyond the scope of this review, but it has been thoroughly reviewed by Diamond et al. (2006a), Reinert et al. (2002), and Gosset et al. (2016). In addition, the EPA also has recommended modeling approaches to determine appropriate criteria

for intermittent exposures (USEPA 1991) and these approaches were are thoroughly discussed and analyzed by Butcher and Diamond (2003).

4.3. Time-Averaged Concentration (TAC)

Time-averaged concentration (TAC) is a measurement of dose experienced by organisms during pulsed exposures that account for pulse duration, frequency, and magnitude. An example equation for TAC is:

$$TAC = (\left[PC \times t_p\right] + RC \times \left[t_d - t_p\right])/t_d$$

Where PC is the mean measured toxicant concentration, t_p is the pulse duration, RC is the concentration of the dissolved toxicant between pulses, and t_d is the total test duration (Angel et al. 2010, 2015, 2017). Note, that in cases where there are multiple pulses (even if they are of different magnitudes), another $PC \times t_p$ can be added to the numerator for each additional pulse (and making sure to account for each additional t_p term in the $t_d - t_p$ term. In addition, Angel et al. (2010, 2015, 2017), accounts for loss of toxicant throughout the pulse period (primarily to binding to the container, etc.) by averaging the toxicant concentration at the beginning of the pulse and immediately prior to a water change, assuming linear decrease in concentration of the contaminant. Angel et al. (2010, 2015, 2017) have found that the TAC does a relatively good job predicting toxicity, regardless of pulse exposure regime for several metal contaminants and a range of freshwater and marine toxicological species (Figure 4-1 Figure 4-1).

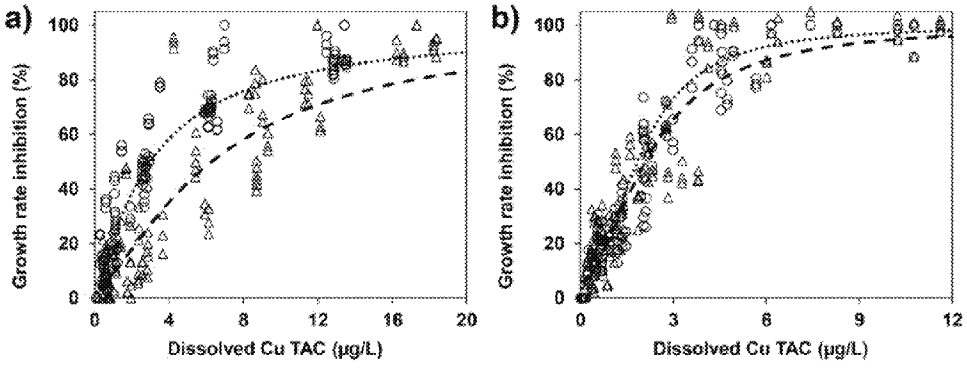

Figure 4-1. Relationship between growth rate inhibition and the time-averaged concentration (TAC) of dissolved copper for continuous (circles) and pulse (triangles) copper exposures to the algae (a) *Pseudokirchneriella subcapitata* and (b) *Chlorella sp.* The dotted and dashed lines show the models used to calculate IC10, 20 and 50's for continuous and pulsed exposure, respectively. (From Angel et al. 2017).

Angel et al. (2017) acknowledges that the TAC calculations better predicted toxicity for *Chlorella sp.* (Figure 4-1~~Figure 4-1~~b) than *P. subcapitata* (Figure 4-1~~Figure 4-1~~a). Several of the factors discussed in Section 3~~3~~ could account for the variability observed with *P. subcapitata*. For example, the somewhat increased toxicity of longer pulses at lower concentrations vs. short pulses and higher concentrations could be explained by copper internalization as a critical burden has to be met to elicit a toxic response (Mancini 1983, Diamond et al. 2006b). In a marine diatom, it was found that when copper concentrations where sufficiently high and pulses were short, uptake was lower because the transport proteins were saturated, and the copper instead bound to the surface of the algae, and was not available for internalization due to its rapid desorption back into the solution (Angel et al. 2015). In addition, other factors such as effects due to organism age and recovery periods (such as contaminants that are slow to depurate) could result in TACs underestimating toxicity. These factors should be considered when assessing whether a TAC approach is appropriate for regulating pulsed exposures (Angel et al. 2017).

In addition to the above-mentioned factors, the TAC approached remains untested in natural waters and there are uncertainties in the extrapolation from the lab to the field, particularly with mixtures of contaminants (Burton et al. 2000, Angel et al. 2017). In addition, TAC calculations require a detailed knowledge of the pulsed regime and chemical concentrations to apply appropriately, which is challenging for any given field situation. However, with the development of passive sampling devices that provide integrated concentrations over their deployment time, such as diffusive gradients in thin films (DGTs) (Davison and Zhang 2012) and Polar Organic Chemical Integrative Samplers (POCIS) (Alvarez 2010), this issue could be mitigated. However, with more testing and an appropriately conservative approach applying TACs, there is promise that this method may have merit as an added tool to support regulatory requirements in the future.

A TAC approach relies on knowing specific chemical concentrations of interest. This tool may benefit development of site-specific water quality but is not applicable to direct whole effluent toxicity testing with unknown mixtures and chemical concentrations.

4.4. Median-Effect Time Criteria

Traditional toxicity has relied on fixed duration tests that vary sample or contaminant concentrations to calculate median effect concentrations (EC50) or no observable effect concentrations (NOEC). Currently, using WET methodologies, the exposure durations are set based on organism life history rather than relevant environmental exposure. While many of the pulsed testing methods attempt to change the exposure to environmentally relevant durations, they are still able to include multiple test concentrations providing a measure of the magnitude of exposure (Stransky et al. 2015, Rosen et al. in press). The following procedure takes a different approach; instead of modulating exposure concentration, it aims to modulate exposure duration to come up with a median lethal effect time (LET50). This procedure of modulating time to effect has been used in other pulsed toxicity testing (Abel and Garner 1986, Brent and Herricks 1998, Andersen et al. 2006). However, unlike traditional LET50 tests, the exposure times for this test

would be discrete, brief, and more representative of wet weather events (Brent and Herricks 1999). This procedure of modulating time to effect has been used in other pulsed toxicity test studies (Abel and Garner 1986, Brent and Herricks 1998, Andersen et al. 2006).

In addition, the procedure proposed by Brent and Herricks (1999) acknowledges the importance of the post exposure period for observation of latency effects (Brent and Herricks 1998, Angel et al. 2010). This method can derive a pulse exposure duration to a sample that produces the 50% effect of the test population during the post-exposure period; a PE-LET50 value (Brent and Herricks 1999). The PE-LET50 for each of several discrete samples taken during a storm event would then be used to calculate an event toxicity unit (ETU). The ETU can be an effective assessment tool and would allow comparisons across monitoring events to determine relative toxicity (Brent and Herricks 1999). Brent and Herricks (1999) used the procedure on two storm sites and for two reference tests and found good agreement with what was expected based on concurrent standard reference tests. They used *Ceriodaphnia dubia* for the test as it demonstrates post-exposure effects relatively quickly (48 h) compared to other test species such as *H. azteca* and *P. promelas* (Brent and Herricks 1998).

Figure 4-2

~~Figure 4-2~~ shows a schematic of the toxicity tests procedure proposed by Brent and Herrick (1999). In short, it consists of four basic steps:

1. Sampling – take several discrete water samples throughout the duration of the event. The interval between samples should be consistent and should reflect the size of the storm so that 10-24 samples are taken.
2. Pre-screening toxicity test – run a short toxicity test on undiluted samples of the stormwater using standard continuous procedures to screen for toxic samples. Samples that are not toxic (i.e. effect less than 50% mortality) will not be tested again, but toxic samples move on to step 3.
3. Run a full, pulsed, toxicity test with each toxic sample using at least four durations (picked for relevance to wet weather event). Include a post-exposure observation period appropriate for the tested species. This test will be used to determine PE-LET50s.
4. Using the calculated PE-LET50s, plot the inverse of the PE-LET50s again the duration of the storm (assign values of 0 for the 1/PE-LET50s of non-toxic samples from step 2). Integrate the plotted curve to determine the ETU.

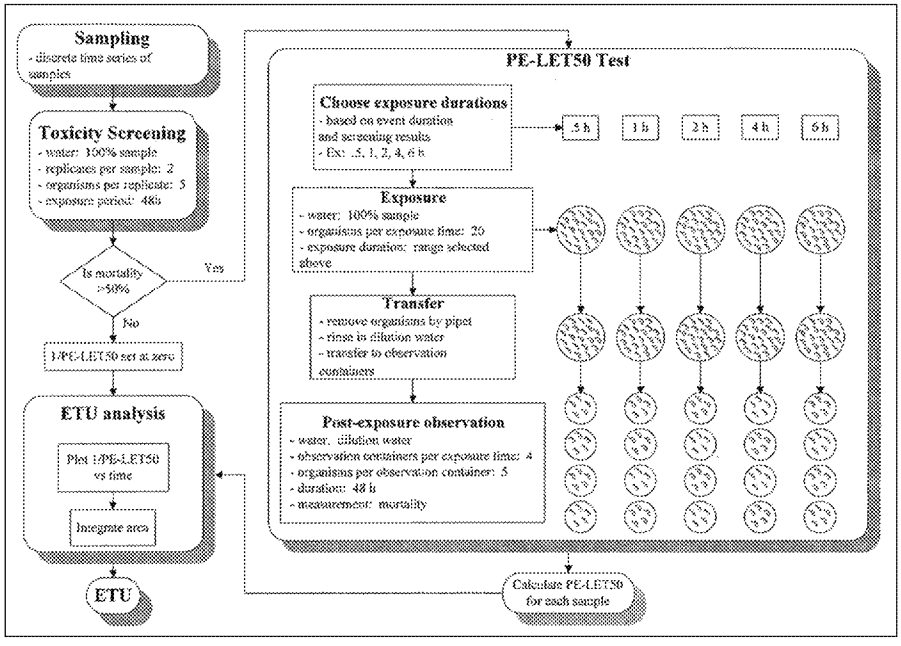

Figure 4-2. Proposed wet weather analysis method using a median effect time-averaged concentration (PE-LET50/ETU) with *C. dubia*. (From Brent and Herricks 1999).

This procedure appears to be quite rigorous and does not rely on specific knowledge of the water chemistry to understand the specific toxicity of a storm. Furthermore, the creation of the ETU makes results of the test easy to compare across storms and locations. Using exposure duration, instead of concentration, is an interesting way to approach determining toxicity and might be more relevant for intermittent storm events. However, storm magnitude may be an important missed factor where runoff from a rain event that lasts 24 hours and amounts to 0.5 inches may have substantially different chemical and physical properties than another rain event that is also 24 h but with 2.5 inches and rainfall. This procedure however, is logistically challenging and time intensive. At the onset of a storm, it is unknown how long it will last, but assuming a flow-weighted composite sample is collected, then there is no need for the extra proposed screening steps. One additional challenge of particular note is that storm water samples would have to be used outside of the current acceptable holding time of 36 h due to running a pre-screening and then full toxicity test. This is one reason why *C. dubia* was chosen for this demonstration; to keep test duration as short as possible to avoid degradation of potential toxicants. Stormwater samples frequently loose toxicity rapidly during holding given rapid changes in physical and chemical properties as the samples settle and age. This procedure also requires a large testing design (each stormwater sample

would consist of 10-24 discrete samples vs. just one flow-averaged composite sample). It is unreasonable to run this procedure on all effluent sources that current NPDES permitting requires to be tested during a storm event. However, there are potentially simpler designs that could be implemented (such as fewer discrete samples, or several composited samples over intervals during the storm) that might produce a similar effect for less effort.

5. Representative Sampling Approaches

Critical to any monitoring and testing program is the ability to collect a representative sample for testing. Because stormwater discharge characteristics often vary dramatically over time, sampling methods are an important aspect of this program. Unless there is high confidence that a discharge has uniform characteristics (e.g. a mixed tank of water or ballast water), it is highly recommended that a composite sample of the discharge be captured for testing purposes. Composite samples may collected throughout the duration of a storm event, or over a given period such as the first few hours of runoff (first-flush). Capturing of first-flush is also often required or recommended to assess the most conservative scenario where pollutants tend to have the greatest concentrations, however, there is currently no clear guidance on what the definition of first-flush is, and there is always the potential that capturing only the first portion of a storm may miss pulses of contaminants that may occur later during a storm after becoming mobilized, particularly true for larger watersheds or catch basins.

Samples may be collected from manual grab samples, or through the use of an auto sampler. They may also be time-weighted or flow-weight composited depending on the circumstance. A time-weighted composite is simpler and acceptable if flow and water quality characteristics are similar over time, or when grab samples are collected at different times from large receiving water bodies. A flow-weighted composite is recommended for discharges that vary substantially over time in both characteristics and flow. In many current cases a single grab sample is often collected to represent an entire episodic event like stormwater runoff, yet studies have shown substantial variability in both chemical concentrations and toxicity at end-of-pipe over the course of any given event (Kayhanian et al. 2008). A primary goal of this ESTCP effort is to develop more representative toxicity testing regimes for episodic discharges, but it is also important to do so using appropriately representative samples, which will be explored through this program with ultimate guidance recommendations.

6. Considerations for Regulatory Acceptance

Despite a majority of research indicating that pulsed exposures better represent conditions experienced by organisms in the receiving environment of intermittent discharges, currently, regulatory agencies require WET testing methods developed specifically for continuous discharges. A primary reason for this is the lack of standardized procedures to apply pulsed exposure methodology in a regulatory framework. To gain regulatory acceptance for any proposed methods to address episodic exposures we must include considerations with regard to both exposure duration and magnitude as discussed among the literature cited in this review document. We ideally can also develop an acceptable framework within which the modified pulsed exposure methods can be used as a stronger line of evidence to assess compliance with beneficial uses in the receiving waters. A more robust and holistic framework should ideally include an assessment of toxicity in both the receiving waters and end of pipe. Careful consideration with regard to the species, exposure duration, and test endpoints must be addressed. For example, acute testing with

pulsed exposures is likely more applicable for end of pipe samples, but simultaneous chronic tests with continuous exposures might be warranted in the receiving water depending on the site. Ultimately the biological communities within the receiving environment should also be evaluated to determine the likelihood of impacts from pulsed exposures. Each of these measures becomes a complimentary line of evidence to better evaluate effects from episodic discharges before significant efforts are expanded on expensive remediation options or potentially fines.

7. Conclusions

Intermittent discharges are common in highly urbanized environments, especially during storm events. Current WET methodologies require continuous exposure to end-of-pipe samples for up to 96 h for acute tests and 7 d for chronic tests. Pulsed exposure methods have been suggested to make toxicity testing methods for these intermittent discharges more relevant to conditions experienced in receiving environments (Burton et al. 2000, Reinert et al. 2002, Diamond et al. 2006b, Gordon et al. 2012, Gosset et al. 2016). However, when designing these protocols, it is critical to consider mechanism of action of the contaminant as well as the organism that is being tested, as evidenced by the discussion in Section 22.

Current research underway at SSC Pacific is considering using two saltwater species, *Strongylocentrotus purpuratus* and *Americamysis bahia,* and two freshwater species *Ceriodaphnia dubia* and *Hyalella azteca* for additional pulsed study exposure evaluations in support of protocol development for episode discharges. Laboratory pulsed exposure tests will be conducted using copper, zinc, and an organic pollutant relevant to stormwater discharges in highly urbanized environments (i.e., benzo (a) pyrene, a common polycyclic aromatic hydrocarbon [PAH]). Copper, zinc, and a PAH have been selected as test chemicals based on their multiple sources and prevalence in stormwater runoff, as well as a variety of toxicity identification evaluation (TIE) studies that have attributed toxicity to these specific chemicals in stormwater runoff (Kayhanian et al. 2008, Katz et al. 2006). Based on this review, here are a couple of considerations:

Commented [HT6]: More than a couple

1. Latent effects are likely as several studies with metals and various organic chemicals demonstrated latent effects with multiple test species including crustaceans. However, studies to date suggest that the post-exposure durations needed to adequately characterize post-exposure effects top out at approximately 120 h for similar crustacean species (Table 3-3~~Table 3-3~~). Based on these observations, standard WET acute test durations of 96-h and chronic durations of 7-days will be performed inclusive of the pulse and a post-exposure observation period. These time frames should be sufficient to capture any latent effects following exposure to the sample of interest.
2. Exposures to pulsed samples will be performed using organisms that are within the recommended range in the standard WET protocols. However, additional studies to be performed at SSC Pacific will evaluate effects on different ages to specifically help interpret data collected from *in situ* validation studies where exposures to pulsed samples of interest (e.g. stormwater plumes) may occur post deployment at an age range exceeding standard protocol requirements.
3. Designing experiments using TAC procedures have proven merit for testing of individual compounds, particularly trace metals given the substantial efforts conducted with them to date (Angel et al. 2010, 2015, 2017). However, the applicability of the

TAC as an easily implemented approach for compliance testing of ambient complex samples has not been demonstrated to date.

4. While methods using median lethal effect time criteria (e.g. LET50) are potentially a useful way to characterize toxicity of pulsed events, it may be easier to focus on median concentration effect criteria (e.g. LC50) as they are more common. However, it should be relatively easy to derive LET50s from some of our tests, especially with mysids, as daily counts are standard practice.

5. The promising approach that we intend to focus most of our efforts on will be simply altering the exposure to a relevant time-period based on site-specific considerations (e.g. 95% runoff duration or the time to empty a dry dock). Following a pulsed exposure, the test organisms are then transferred to receiving water from the monitored location, or clean lab water if an adjacent receiving water is not available. Dilutions may be incorporated to add a level of realism that represents the mixing zone in the receiving waters, thus incorporating both time of exposure and magnitude for a more accurate assessment compared to existing continuous exposure methods.

Commented [LJ7]: I would add a different exposure scenario to this. Since environmentally relevant exposures are not all or nothing and transferring the organisms several times during a 4-7 days test may add stress, considering using a renewal technique that removes 80-90% of the 100% sample and replacing it with control water. Then renew that again in the same manner 8-12hrs later.

Commented [LJ8]: I recommend adding two more testing conditions 6) Representative flow weighted composite sampling and testing and 7) %mixing zone testing based on a 28 hrs rain event.

6.

Based on extensive literature, and the limited research using stormwater samples to test pulsed exposure methods (Dupuis and Kreutzberger 2003, Rosen et al. in press), pulsed exposure toxicity testing methodologies have proven their ability to provide more appropriate and relevant methods to more accurately assess toxicity and potential ecological effects in associated receiving waters related to episodic discharges. This literature review provides confidence that a modified toxicity testing regime is warranted and feasible for routine compliance monitoring of a variety of episodic discharge scenarios.

Better, more representative methods are needed to make such important decisions on and to also to mitigate liability risk where not warranted. We also recognize the need to better incorporate toxicity test results into a more comprehensive assessment of episodic discharges and associated receiving water quality. Ultimately, the test method itself is just one line of evidence in the toolbox. Through this process a goal for this program will be to also help devise a multiple-line of evidence approach that can be used for appropriate decision making such as the need for and proper types of BMPs. There is also a well-recognized need for guidance on more representative sampling methods for episodic discharge that we anticipate this program assisting with as well.

8. References

Abel, P.D., and S.M. Garner. 1986. Comparisons of median survival times and median lethal exposure times for *Gammarus pulex* exposed to cadmium, permethrin and cyanide. Water Research, 20:579–582.

Alvarez, D.A. 2010. Guidelines for the use of the semipermeable membrane device (SPMD) and the polar organic chemical integrative sampler (POCIS) in environmental monitoring studies: U.S. Geological Survey, Techniques and Methods 1–D4, 28 p. (http://pubs.usgs.gov/tm/tm1d4/.)

Andersen, T.H., R. Tjørnhøj, L. Wollenberger, T. Slothuus, and A. Baun. 2006. Acute and chronic effects of pulse exposure of Daphnia magna to dimethoate and pirimicarb. Environmental Toxicology and Chemistry, 25:1187–1195.

Angel, B.M., S.L. Simpson, A.A. Chariton, J.L. Stauber, and D.F. Jolley. 2015. Time-averaged copper concentrations from continuous exposures predicts pulsed exposure toxicity to the marine diatom, *Phaeodactylum tricornutum*: Importance of uptake and elimination. Aquatic Toxicology, 164:1–9.

Angel, B.M., S.L. Simpson, E. Granger, K. Goodwyn, and D.F. Jolley. 2017. Time-averaged concentrations are effective for predicting chronic toxicity of varying copper pulse exposures for two freshwater green algae species. Environmental Pollution, 230:787–797.

Angel, B.M., S.L. Simpson, and D.F. Jolley. 2010. Toxicity to *Melita plumulosa* from intermittent and continuous exposures to dissolved copper. Environmental Toxicology and Chemistry, 29:2823–2830.

Bailey, H.C., J.L. Miller, M.J. Miller, L.C. Wiborg, L. Deanovic, and T. Shed. 1997. Joint acute toxicity of diazinon and chlorpyrifos to *Ceriodaphnia dubia*. Environmental Toxicology and Chemistry, 16:2304–2308.

Bearr, J.S., J. Diamond, H. Latimer, and M. Bowersox. 2006. Effects of pulsed copper exposures on early life-stage *Pimephales promelas*. Environmental Toxicology and Chemistry, 25:1376–1382.

Brent, R., and E. Herricks. 1998. Post exposure Effects of Brief Cadmium, Zinc, and Phenol Exposures on Freshwater Organisms. Environmental Toxicology, 17:2091–2099.

Brent, R.N., and E.E. Herricks. 1999. A method for the toxicity assessment of wet weather events. Water Research, 33:2255–2264.

Burton, G.A., R. Pitt, and S. Clark. 2000. The Role of Traditional and Novel Toxicity Test Methods in Assessing Stormwater and Sediment Contamination. Source Critical Reviews in Environmental Science and Technology, 30:413–447.

Butcher, J.B., and J.M. Diamond. 2003. Effluent limits for fluctuating discharges: alternate frameworks for water quality criteria incorporating chemical frequency, magnitude, and duration. Report 98-HHE-3, Water Environment Research Foundation, Alexandria, VA.

Butcher, J., J. Diamond, J. Bearr, H. Latimer, T.S.J. Klaine, T. Hoang, and M. Bowersox. 2006. Toxicity models of pulsed copper exposure to *Pimephales promelas* and Daphnia magna. Environmental Toxicology and Chemistry, 25:2541–2550.

Colvin, M., G. Rosen, N.T. Hayman, and K. Kowal. in prep. Historical Rainfall Analysis. Project Deliverable for Project# ER-201727.

Davison, W., and H. Zhang. 2012. Progress in understanding the use of diffusive gradients in thin films (DGT) back to basics. Environmental Chemistry, 9:1–13.

de Zwart D., Adams W., Burgos M.G., Hollender J., Junghans M., Merrington G., Muir D., Parkerton T., De Schamphelaere K., Whale G., Williams R. 2017. Aquatic exposures of chemical mixtures in urban environments: approaches to impact assessment. Environmental Toxicology and Chemistry, 37(3):703-714. DOI: 10.1002/etc.3975.

Diamond, J. and C. Daley. 2000. What is the relationship between whole effluent toxicity and instream biological condition? Environmental Toxicology and Chemistry, 19:158-168.

Diamond, J., M. Bowersox, H. Latimer, C. Barbour, J. Bearr, and J. Butcher. 2005. Effects of pulsed contaminant exposures on early life stages of the fathead minnow. Archives of Environmental Contamination and Toxicology, 49:511–519.

Diamond, J.M., J.B. Butcher, and S.J. Klaine. 2006a. Factoring frequency, magnitude, and duration in NPDES permit conditions. Report 02-WSM-3, Water Environment Research Foundation, Alexandria, VA.

Diamond, J.M., S.J. Klaine, and J.B. Butcher. 2006b. Implications of pulsed chemical exposures for aquatic life criteria and wastewater permit limits. Environmental Science and Technology, 40:5132–5138.

Diamond, J., J. Stribling, M. Bowersox, and H. Latimer. 2008. Evaluation of Effluent Toxicity as an Indicator of Aquatic Life Condition in Effluent-Dominated Streams: A Pilot Study. Integrated Environmental Assessment and Management, 4: 456–470.

Diamond, J., R. Altenburger, A. Coors, S. Dyer, M. Focazio, K. Kidd, A. Koelmans, K. Leung, M. Servos, J. Snape, J. Tolls, and X. Zhang. 2017. Use of prospective and retrospective risk assessment methods that simplify chemical mixtures associated with treated domestic wastewater discharges. Environmental Toxicology and Chemistry, 37(3):690-702. DOI: 10.1002/etc.4013.

Dupuis, T.V, and W.A. Kreutzberger. 2003. Wet weather discharges: use of time-variable toxicity testing in a decision-solution framework. Proceeding of the Water Environment Federation, WEFTEC 2003: Session 1 -10, pp. 831-849 (19).

Early P.J., Rosen G., Rivera-Duarte I., Gauthier R.D., Arias-Thode Y., Thompson J., Swope B. 2007. A Comprehensive Copper Compliance Strategy: Implementing Regulatory Guidance at Pearl Harbor Naval Shipyard & Intermediate Maintenance Facility. SSC Pacific Technical Report #1952. January 2007, 231pp. http://www.dtic.mil/dtic/tr/fulltext/u2/a464523.pdf

Fisher, D.J., D.T. Burton, L.T. Yonkos, S.D. Turley, B.S. Turley, G.P. Ziegler, and E.J. Zillioux. 1994. Acute and short- term chronic effects of continuous and intermittent chlorination on *Mysidopsis bahia* and *Menidia beryllina*. Environmental Toxicology and Chemistry, 13:1525–1534.

Forbes, V.E., A. Palmqvist, and L. Bach. 2006. The Use and Misuse of Biomarkers in Ecotoxicology. Environmental Toxicology and Chemistry, 25:272-280.

Gama-Flores, J.L., S.S.S. Sarma and S. Nandini. 2007. Exposure time-dependent cadmium toxicity to *Moina macropa* (Cladocera); a life table demographic study. Aquatic Ecology, 41:639-648.

Gordon, A.K., S.K. Mantel, and N.W. J. Muller. 2012. Review of toxicological effects caused by episodic stressor exposure. Environmental Toxicology and Chemistry, 31:1169–1174.

Gosset, A., Y. Ferro, and C. Durrieu. 2016. Methods for evaluating the pollution impact of urban wet weather discharges on biocenosis: A review. Water Research, 89:330–354.

Handy, R.D. 1994. Intermittent exposure to aquatic pollutants: assessment, toxicity and sublethal responses in fish and invertebrates. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology, 107:171–184.

Heming, T.A., E.J. Mcguinness, L.M. George, and K.A. Blumhagen. 1988. Effects of Pulsed-Exposure and Spiked-Exposure to Methoxychlor on Early Life Stages of Rainbow-Trout. Bulletin of Environmental Contamination and Toxicology, 40:764–770.

Hoang, T.C., J.S. Gallagher, J.R. Tomasso, and S.J. Klaine. 2007a. Toxicity of two pulsed metal exposures to *Daphnia magna*: Relative effects of pulsed duration-concentration and influence of interpulse period. Archives of Environmental Contamination and Toxicology, 53:579–589.

Hoang, T.C., and S.J. Klaine. 2007. Influence of organism age on metal toxicity to *Daphnia magna*. Environmental Toxicology and Chemistry, 26:1198–1204.

Hoang, T.C., and S.J. Klaine. 2008. Characterizing the toxicity of pulsed selenium exposure to Daphnia magna. Chemosphere, 71:429–438.

Hoang, T.C., J.R. Tomasso, and S.J. Klaine. 2007b. an Integrated Model Describing the Toxic Responses of Daphnia Magna to Pulsed Exposures of Three Metals. Environmental Toxicology and Chemistry, 26:132.

Hoang, T., J.S. Gallagher, and S.J. Klaine. 2007c. Responses of *Daphnia magna* to pulsed exposures of arsenic. Environmental Toxicology, 22:308–317.

Hogan, A.C., M.A. Trenfield, A.J. Harford, and R.A. Van Dam. 2013. Toxicity of magnesium pulses to tropical freshwater species and the development of a duration-based water quality guideline. Environmental Toxicology and Chemistry, 32:1969–1980.

Holdway, D.A., and D.G. Dixon. 1985. Acute toxicity of pulse-dosed methoxychlor to juvenile American flagfish (*Jordanella floridae goode* and *bean*) as modified by age and food availability. Aquatic Toxicology, 6:243–250.

Holdway, D.A., and D.G. Dixon. 1988. Acute toxicity of permethrin or glyphosate pulse exposure to larval white sucker (*catostomus commersoni*) and juvenile flagfish (*Jordanella floridae*) as modified by age and ration level. Environmental Toxicology and Chemistry, 7:63–68.

Holth, T.F., R. Nourizadeh-Lillabadi, M. Blaesbjerg, M. Grung, H. Holbech, G. I. Petersen, P. Aleström, and K. Hylland. 2008. Differential gene expression and biomarkers in zebrafish (*Danio rerio*) following exposure to produced water components. Aquatic Toxicology, 90:277–291.

Hosmer, A., L. Warren, and T. Ward. 1998. Chronic toxicity of pulse-dosed fenoxycarb to *Daphnia magna* exposed to environmentally realistic concentrations. Environmental Toxicology, 17:1860–1866.

Kallander, D.B., S.W. Fisher, and M.J. Lydy. 1997. Recovery following pulsed exposure to organophosphorus and carbamate insecticides in the midge, *Chironomus riparius*. Archives of Environmental Contamination and Toxicology, 33:29–33.

Katz, C., G. Rosen, and E. Arias. 2006. Storm water toxicity evaluation conducted at Naval Station San Diego, Naval Submarine Base San Diego, Naval Amphibious Base Coronado, and Naval Air Station North Island. San Diego, CA. SSC Pacific Technical Report #1938. May 2006, 715pp. http://www.dtic.mil/dtic/tr/fulltext/u2/a451054.pdf

Kayhanian, M., C. Stransky, S. Bay, S. L. Lau, and M.K. Stenstrom. 2008. Toxicity of urban highway runoff with respect to storm duration. Science of the Total Environment, 389:386–406.

Kim, Y., J. Jung, S. Oh, and K. Choi. 2008. Aquatic toxicity of cartap and cypermethrin to different life stages of *Daphnia magna* and *Oryzias latipes*. Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes, 43:56–64.

Laurén, D.J., and D.G. McDonald. 1987. Acclimation to Copper by Rainbow Trout, *Salmo gairdneri*: Physiology. Canadian Journal of Fisheries and Aquatic Sciences, 44:99–104.

Lynch, N.R., T.C. Hoang, T.F. O'Brien. 2016. Acute toxicity of binary-metal mixtures of copper, zinc, and nickel to *Pimephales promelas*. Evidence of more-than-additive effect. Environmental toxicology and chemistry 35: 446-457.

Mancini, J.L. 1983. A method for calculating effects, on aquatic organisms, of time varying concentrations. Water Research, 17:1355–1362.

McCahon, C.P., M.J. Poulton, P.C. Thomas, Q. Xu, D. Pascoe, and C. Turner. 1991. Lethal and sub-lethal toxicity of field simulated farm waste episodes to several freshwater invertebrate species. Water Research, 25:661–671.

Milne, I., J. Seager, and M. Mallett. 2000. Effects of short-term pulsed ammonia exposure on fish. Environmental Toxicology and Chemistry, 19:2929–2936.

Naddy, R.B., K.A. Johnson, and S.J. Klaine. 2000. Response of *Daphnia Magna* to Pulsed Exposures of Chlorpyrifos. Environmental Toxicology and Chemistry, 19:423–431.

Formatted: Normal, Indent: Before: 0", Hanging: 0.25"

Formatted: Font: Font color: Custom Color(RGB(34,34,34))

Naddy, R.B., and S.J. Klaine. 2001. Effect of pulse frequency and interval on the toxicity of chlorpyrifos to *Daphnia magna*. Chemosphere, 45:497–506.

Perez, E., T.C. Hoang. 2017. Chronic toxicity of binary-metal mixtures of cadmium and zinc to *Daphnia magna*. Environmental toxicology and chemistry 36:2739-2749

Perez, E., T.C. Hoang. 2018. Responses of *Daphnia magna* to chronic exposure of cadmium and nickel mixtures. Chemosphere 208:991-1001

Phillips, B.M., P.A. Nicely, J.W. Hunt, B.S. Anderson, R.S. Tjeerdema, S.E. Palmer, F.H. Palmer, and H.M. Puckett. 2003. Toxicity of cadmium-copper-nickel-zinc mixtures to larval purple sea urchins (*Strongylocentrotus purpuratus*). Bulletin of Environmental Contamination and Toxicology, 70:592–599.

Posthuma, L., C. Brown, D.de Zwart, J. Diamond, S. Dyer, M. Hamer, C. Holmes, S. Marshall, and A. Burton. 2017. Simplifying environmental mixtures – an aquatic exposure-based approach via land use scenarios. Environmental Toxicology and Chemistry, 37:671-673.

Pynnönen, K. 1990. Aluminum accumulation and distribution in the freshwater clams (*unionidae*). Comparative Biochemistry and Physiology Part C: Comparative Pharmacology, 97:111–117.

Reinert, K.H., J.M. Giddings, and L. Judd. 2002. Effects analysis of time-varying or repeated exposures in aquatic ecological risk assessment of agrochemicals. Environmental Toxicology and Chemistry, 21:1977–1992.

Rosen, G., M. Colvin, C. Katz, J. Munson-Decker, and N.T. Hayman. In press. Pulsed Exposure Toxicity Testing: Method Development and Initial Evaluation for Stormwater Compliance. San Diego. SSC Pacific Technical Report #TBD.

Rosenkrantz, R.T., C.A. Pollino, D. Nugegoda, and A. Baun. 2008. Toxicity of water and sediment from stormwater retarding basins to *Hydra hexactinella*. Environmental Pollution, 156:922–927.

Schiff, K., S. Bay, and D. Diehl. 2003. Stormwater toxicity in Chollas Creek and San Diego Bay, California. Environmental Monitoring and Assessment, 81:119–132.

Sharma, S.S., H. Schat, R. Vooijs, and L.M. Van Heerwaarden. 1999. Combination toxicology of copper, zinc, and cadmium in binary mixtures: Concentration-dependent antagonistic, nonadditive, and synergistic effects on root growth in *Silene vulgaris*. Environmental Toxicology and Chemistry, 18:348–355.

Stephan, C., I. Mount, D. Hansen, J. Gentile, G. Champan, and W. Brungs. 1985. Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses. Washington DC.

Stransky, C., K. Tait, P. Arth, A. Cibor, and R. Kolb. 2015. Pulsed Salinity, Pyrethroid, and Copper Study – Final Report. Prepared by Amec Foster Wheeler for the City of San Diego Transportation and Storm Water Department. May 2015.*copy of report will be provided upon request of authors*

Formatted: Normal, Indent: Before: 0", Hanging: 0.25"

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font color: Custom Color(RGB(34,34,34))

Timourian, H. 1968. The effect of zinc on sea urchin morphogenesis. Journal of Experimental Zoology, 169:121–131.

US EPA. 1991. Technical support document for water quality-based toxic control, U.S. Environmental Protect Agency, Office of Water, Washington DC. EPA/505/2-90/001.

US EPA. 1972. Federal Water Pollution Control Act Amendments of 1972. 33 U.S.C. §1251 et seq. (1972), http://www2.epa.gov/laws-regulations/summary-clean-water-act.

US EPA. 1995. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms. U.S. Environmental Protection Agency, Office of Research and Development, Cincinnati, Ohio. EPA/600/R-95/136.

US EPA. 2002a. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, U.S. Environmental Protection Agency, Office of Water, Washington DC. EPA/821/R-02/012.

US EPA. 2002b. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, U.S. Environmental Protection Agency, Office of Water, Washington DC. EPA/821/R-02/013.

US EPA. 2002c. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. U.S. Environmental Protection Agency, Office of Water, Washington DC. EPA/600/R-95/136.

Verslycke, T., M. Vangheluwe, D. Heijerick, K. De Schamphelaere, P. Van Sprang, and C.R. Janssen. 2003. The toxicity of metal mixtures to the estuarine mysid *Neomysis integer* (Crustacea: *Mysidacea*) under changing salinity. Aquatic Toxicology, 64:307–315.

Widianarko, B., F.X.S. Kuntoro, C.A.M. Van Gestel, and N.M. Van Straalen. 2001. Toxicokinetics and toxicity of zinc under time-varying exposure in the guppy *(Poecilia reticulata)*. Environmental Toxicology and Chemistry, 20:763–768.

Williams, N.D., and D.A. Holdway. 2000. The effects of pulse-exposed cadmium and zinc on embryo hatchability, larval development, and survival of Australian crimson spotted rainbow fish (*Melanotaenia fluviatilis*). Environmental Toxicology, 15:165–173.

Zahner, H.M., E.J. VanGenderen, J.R. Tomasso, and S.J. Klaine. 2006. Whole-body sodium concentration in larval fathead minnows (*Pimephales promelas*) during and following copper exposure. Environmental Toxicology and Chemistry, 25:1635–1639.

Zhao, Y., and M.C. Newman. 2006. Effects of exposure duration and recovery time during pulsed exposures. Environmental Toxicology and Chemistry, 25:1298–304.